METABOLISM OF WOMEN DURING THE REPRODUCTIVE CYCLE

VI. A CASE STUDY OF THE CONTINUOUS NITROGEN UTILIZATION OF A MULTIPARA DURING PREGNANCY, PARTURITION, PUERPERIUM AND LACTATION

HELEN A. HUNSCHER, FRANCES COPE HUMMELL, BETTY NIMS ERICKSON AND ICIE G. MACY

Research Laboratory of the Children's Fund of Michigan, Detroit

THREE FIGURES

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INTRODUCTION

Many types of observations have been made in this laboratory during the past 12 years that have shown significant individual differences in metabolic processes of healthy, nonpregnant, pregnant, lactating and post-lactating women even when the subjects were studied under apparently similar dietary, environmental and experimental conditions. These individual fluctuations have been reflected not only in the metabolic balances of pregnancy and lactation but in the quantity of milk flow, the chemical composition and biological properties of the milk, the constituents of the blood, the voluntary choice and quantity of food consumed, the physical state and emotional stability of the individual. In view of these disparate findings we have emphasized on numerous occasions that single metabolic balance periods of 4 or even 10 days' duration, conducted at specific intervals during the progression of the reproductive cycle, have unquestionable usefulness in approximating food utilization and evaluating metabolic adjustments, providing a suffi-

ciently large number of observations are obtained. Due, however, to a multiplicity of determinate factors that do not lend themselves to control, data accumulated over brief intervals should not always be considered as representing physiological constants peculiar to a given stage of reproduction of women in general (Macy, Hunscher, Nims and McCosh, '30; Hunscher, Donelson, Nims, Kenyon and Macy, '33). In view of the above facts it is expedient then to extend, not only the short balance to a longer period of time, but to determine uninterruptedly the metabolic responses of the same mother during the development of the fetus and the physiological preparation of her own body for lactation, and thereafter, to extend these observations into parturition, puerperium and lactation thereby learning more specifically where the stresses and strains of child-bearing and child-rearing lay. Such a continuous evaluation, even in the case of one healthy woman during the reproductive cycle as a whole will render service since, to our knowledge, no such information exists at the present time.

Hoffström's ('10) classical observation showed, in a normal woman studied continuously from the seventeenth week of pregnancy to term, that the maternal tissues were enriched during pregnancy beyond the needs of the fetus thus resulting in an appreciable accumulation of 'rest material.' Subsequently, the metabolic balances made in this laboratory (Hunscher, Donelson, Nims, Kenyon and Macy, '33) on the same individuals at intervals during both pregnancy and lactation in multiparae indicated appreciable storage in pregnancy on the one hand, but on the other, losses in some cases from the maternal body in lactation. In view of the urgent want for knowledge on the same individual uninterruptedly in gestation and the subsequent lactation, it is significant to both infant and maternal welfare to measure the total physiological effect of reproduction upon the maternal tissues and to determine whether the losses occurring in parturition, puerperium and early lactation exceed the storage acquired during pregnancy, and thereby cause an actual and conse-

quential drain upon the maternal body, or whether the maternal body is left enriched. As a contribution to the pertinent need for more detailed information on the chemical exchanges[1] taking place during the reproductive cycle of a healthy woman, the present paper records continuous metabolic balance data on the progressive nitrogen utilization of a multipara during 21 weeks of gestation through delivery and puerperium to the eighth week postpartum, a total observation period of approximately 8 months; for comparative purposes data collected from balances made at intervals during the two preceding reproductive cycles are included. For the sake of brevity only references that are pertinent to a succint presentation of the immediate data on human subjects are included since they have been adequately reviewed in recent literature.

EXPERIMENTAL

Subject. An American woman (L.R.)[2] of Scotch-Irish descent, who had already been under the constant observation of this laboratory staff for more than 5 years, including the duration of two uneventful and successful lactations and one pregnancy, again made herself available for continuous metabolic observations during the early part of her fourth reproductive cycle. She enjoyed buoyant health at all times. Frequent medical examinations[3] over a period of years showed her to have an excellent record and to be free from metabolic disturbances. Her normal weight approximated 135 pounds, which is satisfactory for a woman in her thirties with a height of 68.5 inches. Table 1 giving her chronological reproductive history shows the frequency of pregnancies, her condition during parturition and the habitual long and heavy milk flow. Except for confinement, the subject remained in her own home and performed the usual household duties in caring for her

[1] The acid-base mineral balances are to be reported in a subsequent paper.

[2] Referred to in previous publications on milk flow, food consumption, chemical and biological properties of milk and isolated metabolic studies as subject L.R. or case VII.

[3] Grateful acknowledgment is made to Dr. A. S. Guimaraes for his interest in the study, his care of the patient and his untiring cooperation.

home and family. The three former children are healthy, mentally alert with intelligent quotients considerably above average, and emotionally equipoised from birth.

Dietary. Since the subject over a period of years had been accustomed to voluntarily selecting, both quantitatively and

TABLE 1

Chronological reproductive history (subject L.R. or VII)

NUMBER OF REPRODUCTIVE CYCLES	1	2	3	4
Pregnancy				
Age, years	30	32	33	37
Last menstrual period	Feb. 27, 1924	Feb. 15, 1926	Nov. 18, 1927	Sept. 25, 1931
Duration, days	252	280	301	280
Albumin	Negative	Negative	Negative	Negative
State of health	Good	Excellent	Excellent	Excellent
Gain in weight, pounds	25	28	26½	26
Parturition				
Date	Nov. 5, 1924	Nov. 20, 1926	Sept. 14, 1928	July 4, 1932
Type of delivery	Normal	Normal	Normal	Normal
Duration of labor, hours	5	6½	6	4½
Condition of subject	Good	Excellent	Excellent	Excellent
Hemorrhage	Slight	Little	Little	Slight
Birth weight of infant, pounds	4.25	8.25	9.25	8.75
Lactation				
Average daily milk secretion, ounces	80	67	78	50
Duration, weeks	60	50	62	40
Voluntary cessation	Jan. 3, 1925	Nov. 10, 1927	Dec. 5, 1929	April 10, 1933
Post lactation, months	.....	1½	1 week	21½

qualitatively, a well-balanced diet, her usual choice of food was not interfered with during the present study. In addition to the usual fruits, vegetables, cereals and meat she took daily 2 quarts of milk in each of which were incorporated 400 U.S.P. units of vitamin D in the form of a cod liver oil concentrate (Vitex)[4] thus insuring a total food intake that was

[4] The authors and subject are indebted to Dr. Bion East of the Vitex Laboratories Inc., Harrison, N. J., for furnishing the fortified cow's milk during this study.

abundant in all the known essential nutritive principles. In this food consumption her caloric intake averaged 3378 cal. (2524 to 4019) in pregnancy and 3400 cal. (2490 to 4324) in lactation.

General procedure. Successive metabolic balance periods each of 5 days' duration were begun in the reproductive cycle at approximately the one hundred and thirty-fifth day of gestation and carried thereafter continuously. The food was weighed before consumption and at the same time one-half by weight of each serving was placed in a container for chemical analyses. The salt and sugar were served separately and accounted for by period. The feces were marked off by carmine and nitrogen determinations were made on a composite sulfuric acid digest of the fresh material to avoid possible losses during drying (Hawk and Bergeim, '31) The daily urine specimens were preserved with toluol and kept in a refrigerator, and at the end of each metabolic balance period aliquots of the daily collections were made up into a composite for chemical analyses. All nitrogen determinations on food, urine and feces were made by the macro Kjeldahl method except for the urine during lactation when the gasometric method of Van Slyke ('26–'27) was used.

During delivery in the hospital[5] all specimens of maternal body losses were collected including those from the linens from which the blood, amniotic fluid, and other excretions were leached out with distilled water and the extract analysed for nitrogen. By the analyses of the irrigation water, blood, placenta and amniotic fluid it was possible to make a reasonably accurate account of the maternal losses during parturition over and beyond that represented by the fetus.

Throughout the puerperium the absorbent pads containing the blood loss and lochia were analysed. Beginning with the third day postpartum as soon as the mammary secretion

[5] The medical, nursing and dietetic staffs of the Florence Crittenton Hospital gave generous cooperation during the 10 days' confinement period. Graduate nursing service was maintained by the laboratory for the continuous care of the patient, collecting samples and making necessary records.

started the milk was completely emptied from both breasts at 4-hour intervals and aliquot samples were collected for the chemical determinations. During the weeks of lactation the breast milk of each complete day's production was obtained by manual expression at regular intervals each day and reserved as part of the metabolic specimens and the remaining milk was given to the baby by bottle and any surplus was sold to the Detroit Mother's Milk Bureau. It is important to note that this mother by means of her hereditary or environmental endowment was capable of providing a generous quantity of breast milk sufficient to promote excellent growth in her former infants and at the same time furnished additional quantities for a variety of other purposes. Moreover, she manually expressed her breast milk during each reproductive cycle and was, therefore, accustomed to lactating in the absence of the suckling influence with no resultant emotional upset. The frequent and complete removal of milk from the mammary glands stimulated a maximum and uniform milk flow, the reputable quality of which was judged by numerous chemical and biological tests.

During the same interval she became used to weighing her own food and accurately collecting the metabolic specimens, and being an intelligent woman with some scientific training she enjoyed having a part in the studies, because she considered them not only of scientific value to others but she herself derived a considerable degree of personal satisfaction from participating in them.

RESULTS AND DISCUSSION

Tables 2 and 3 give a summary of the data accumulated on the continuous nitrogen retention of one woman in her fourth reproductive cycle. The quantity of nitrogen taken into the body over a 7-month period including the final 145 days of pregnancy, parturition and 53 days after delivery during heavy milk flow, the paths of excretion in the urine, feces and breast milk and the maternal gain or loss are recorded. In addition, the data are supplemented by results from indi-

TABLE 2

Continuous daily nitrogen balances during pregnancy of subject L.R.

DAYS OF PREGNANCY	WEIGHT OF SUBJECT	NITROGEN INTAKE	NITROGEN OUTGO		NITROGEN BALANCE		
			Urine	Feces	Net	Fetus and adnexa[1]	Maternal
	kilo	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
135–141	71.2	19.55	13.84	1.96	+ 3.75	0.13	3.62
142–148	71.7	19.21	14.66	2.22	+ 2.33		2.20
149–155	72.0	17.76	13.38	1.94	+ 2.44		2.31
156–162	72.3	18.17	13.46	2.06	+ 2.65	0.23	2.42
163–167	72.6	18.83	14.09	2.30	+ 2.44		2.21
168–170	73.0	15.80	13.06	1.80	+ 0.94		0.71
171–175	73.3	21.42	12.73	2.09	+ 6.60		6.37
176–180	73.8	19.02	15.40	1.68	+ 1.94		1.71
181–185	74.3	19.86	15.39	2.00	+ 2.47	0.39	2.08
186–190	74.7	15.51	12.86	1.73	+ 0.92		0.53
191–195	75.1	22.33	14.69	3.13	+ 4.51		4.12
196–200	75.3	18.34	13.26	1.84	+ 3.24		2.85
201–205	75.6	16.95	12.28	1.81	+ 2.86		2.47
206–210	75.9	20.06	13.57	1.89	+ 4.60	0.22	4.38
211–215	76.2	21.25	15.44	1.59	+ 4.22		4.00
216–220	76.5	20.12	14.97	1.80	+ 3.35		3.13
221–225	76.8	21.10	13.61	2.05	+ 5.44		5.22
226–230	77.0	19.12	14.02	2.01	+ 3.09		2.87
231–235	77.3	18.21	14.62	1.95	+ 1.64	0.71	0.93
236–240	77.5	19.26	15.07	1.96	+ 2.23		1.52
241–245	77.7	18.45	13.80	1.58	+ 3.07		2.36
246–250	77.9	19.52	15.23	2.42	+ 1.87		1.16
251–255	78.0	18.92	14.30	1.70	+ 2.92		2.21
256–260	78.1	18.80	14.15	1.67	+ 2.98	1.18	1.80
261–265	78.2	18.34	13.56	1.93	+ 2.85		1.67
266–270	78.3	17.40	12.26	1.94	+ 3.20		2.02
271–275	78.4	18.76	14.02	1.44	+ 3.30		2.12
276–280	78.5	19.45	13.76	2.38	+ 3.31		2.13
Mean		19.01			+ 3.06		2.60
Total gain	7.3				446.2		

Day of delivery	18.05 food	5.84 urine	
		0.40 feces	
		25.75 blood	
		20.11 placenta	
		20.28 blood from placenta	
		0.08 amniotic fluid	
		0.24 vomitus	
		72.70 total outgo	— 54.65 balance

[1] The average figures were calculated from a previous review (6).

vidual short time nitrogen balance periods at intervals in the second lactation and during the third cycle including post-lactation (figs. 1 and 2).

Pregnancy. The data on nitrogen retention during pregnancy are given in table 2 which shows the intake, paths of outgo and the net and maternal balance. The latter was

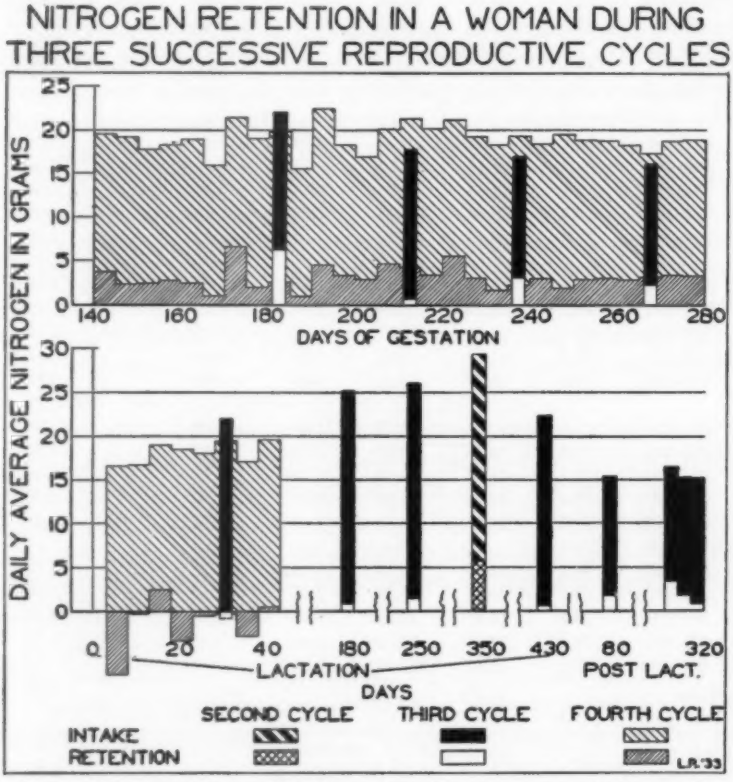

Fig. 1 Continuous nitrogen intake and retention of a woman during the last 145 days of pregnancy through puerperium to the eighth week postpartum in her fourth reproductive cycle upon which are superimposed isolated metabolic balances that were made on the same subject during the two preceding cycles. The average daily grams of nitrogen are plotted on the vertical scale and the days in the periods of the reproductive cycles are given on the horizontal scale.

calculated by the difference between intake and the sum of the excretions and fetal needs. With an average ingestion of 19 gm. (15.8 to 22.3 gm.) of nitrogen from proteins of excellent quality as derived from 2 quarts of milk daily and a well-balanced diet there was a resultant mean net retention of 3.1 gm. (0.92 to 6.6 gm.) per day, thus representing a storage of 16 per cent of the nitrogen intake during the last 145 days of the fourth pregnancy. The ability of the present subject to retain an abundance of nitrogen was further evidenced by the fact that she was able to store an average of 2.6 gm. of nitrogen daily beyond the calculated daily acquisi-

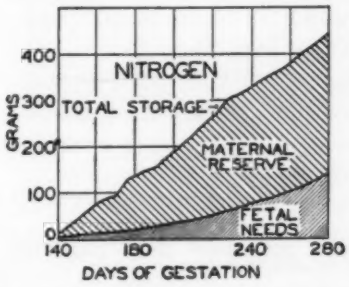

Fig. 2 Determined maternal and estimated fetal storage of nitrogen during the final 145 days of gestation. The maternal retention greatly exceeds the progressive needs of the fetus and its adnexa.

tion of the fetus which weighed 3.7 kg. at term and its adnexa (Macy and Hunscher, '34). It is significant to note that these continuous observations are in agreement with the findings from four metabolic nitrogen balances of 5 days' duration each that were made at monthly intervals in the last half of the third pregnancy when a diet similarly abundant in nitrogen and calories resulted in an average daily net storage of 2.89 gm. (fig. 1).

In the present study the highest retention occurred with the greatest nitrogen intake, but this did not always follow. Two-thirds of the net nitrogen balances were concentrated between 2 and 3.5 gm. retention per day with their intakes varying from

17 to 20 gm. In a previous report (Hunscher, Donelson, Nims, Kenyon and Macy, '33) where the data from the literature on nitrogen requirements of pregnancy and lactation have been assembled, it was shown that of the data from 954 daily balances 48 per cent fell within the range of 1 to 3 gm. of nitrogen retention, figure 3. Furthermore, 80 per cent of the intakes extended from 10 to 18 gm. per day while the retentions came within the range of 1.5 to 2.7 gm. The average daily retention throughout pregnancy from this compilation was 2.28 gm. while for the present subject under continuous

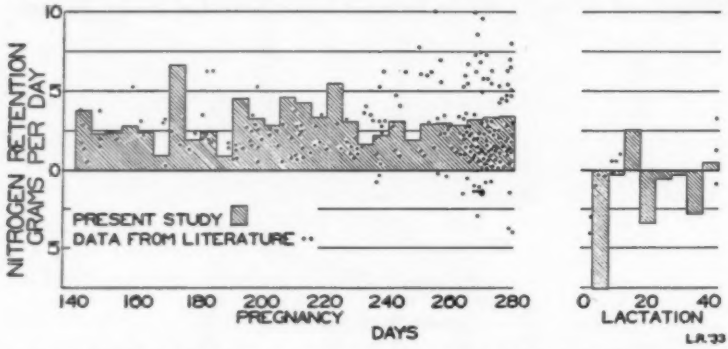

Fig. 3 Nitrogen retentions in grams per day of the present continuous study in pregnancy and lactation upon which are superimposed the scattered nitrogen balances upon women that are available in the literature.

observation it amounted to 3.1 gm. On comparison of the two sets of data, it seems that, in general, the richer the diet in protein and that of good biological values such as from milk and its products, the greater was the persistent storage of nitrogen during pregnancy. These indications corroborate the conclusions of Coons and co-workers (Coons and Blunt, '30; Coons and Marshall, '34; Coons, '35). The physiological advantage of a generous store of nitrogen throughout gestation seems evident, but additional clinical demonstrations are needed.

In pregnancy the amount of tissue building constituents stored in the gravid individual depends upon, 1) the usual

wear and tear of maintenance requirements of the adult individual, 2) the developing fetus and its adnexa, 3) the preparation of the maternal body for parturition and lactation throughout a building up of an excess body store including the hypertrophy of many maternal organs notably of the breasts and uterus, 4) the filling in of the nutritive reserves that perchance were existent through a previous state of undernutrition, and in some cases, 5) upon the growth of the maternal body itself, and 6) the quality and quantity of food.

The total accumulation of nitrogen in the maternal body during the last 145 days of prenatal development amounted to 446 gm. This storage was greater than that reported by Hoffström ('10) from observations begun 2 weeks prior in the term of pregnancy and made continuously to delivery. He found an average daily storage of 1.84 gm. resulting in a total accretion of 310 gm. at term from which were taken 101.5 gm. for calculated fetal needs, leaving 208.5 gm. of 'rest material.' Slemons ('04) and Wilson ('16) found similar amounts. In this study the calculated fetal and maternal needs including estimated utilization of nitrogen in the mammary glands and uterus were estimated to be 146 gm. leaving a remainder of 300 gm. of 'rest material' acquired during the last half of pregnancy by the subject under investigation, figure 2. This abundant storage of maternal nitrogen may be accounted for either by an unusually generous food consumption, or a somewhat depleted maternal body produced by frequent pregnancies followed by long and heavy lactation, and finally, a physiological preparation for an oncoming heavy milk production period. Subject L.R. did not experience any nausea in early pregnancy and it is quite possible that her total nitrogen storage accompanying the gestation period was considerable during the early part of the term before chemical studies were begun. The total gain in body weight during the last 145 days of pregnancy amounted to only 7.3 kg. but the retention of 446 gm. of nitrogen in terms of muscle weight would approximate 14 kg. calculating muscle as 20 per cent protein. It is evident that much remains to be learned of the

fate of the nitrogen retained during pregnancy in the maternal preparation for parturition and the physiological needs of lactation as well as the factors governing the composition and shifts in body weight changes and in addition, the distribution between the maternal body and the fetus on the one hand and breast milk on the other.

Parturition. Parturition and labor made the first toll on the maternal reserve accumulated during pregnancy. The total chemically analyzed loss through urine, feces, placenta, blood, vomitus and amniotic fluid during delivery amounted to 5.84, 0.40, 20.11, 46.03, 0.24 and 0.08 gm. of nitrogen, respectively, yielding a total known loss of 72.70 gm. or 54.65 gm. of nitrogen in excess of the food ingested (table 2). In addition, the infant weighed 3.7 kilos at birth and if its body composition were equivalent to the average figure obtained through the analyses of nine fetuses at term (Macy and Hunscher, '34) it contained approximately 58.6 gm. of nitrogen. The total outgo of nitrogen from the maternal body including the urine, feces, placenta, blood and amniotic fluid and the full term fetus beyond the food consumed amounted to approximately 113 gm. on the day of delivery which left a maternal capital of 333 gm. of nitrogen for future dissipation during puerperium and lactation.

Puerperium. The metabolic nitrogen balance data for the 9 days' postpartum spent in the hospital are shown in table 3. Although the daily food intake was generous with an average of 16.4 gm. (14.4 to 18.5 gm.) of nitrogen, there was a total loss during the 9 days of 44.6 gm. varying from + 2 to — 7.6 gm. of nitrogen per day. This loss during puerperium is proportionate to the results obtained by other investigators (Slemons, '04; Wilson, '16; Mellanby, '12–'13; Harding and Montgomery, '27; and Shaffer, '08–'09). Whether this large nitrogen loss and the creatinuria of puerperium are referable primarily to the involuting uterus or in part to the physiological readjustment from the prenatal state to the inception of lactation or to both is still an open question.[6, 7]

[6] An intensive metabolic study will soon be published on a young primipara.

[7] A detailed consideration of the nitrogen partition of the urine and milk will be reported later.

Slemons ('04) reported that a gravid uterus contained approximately 39 gm. of nitrogen. From the maternal 'rest nitrogen' of 333 gm. that were left after the day of delivery in the present study, the first 9 days of the puerperium made an additional toll which included the inestimable losses from

TABLE 8

Continuous daily nitrogen balances during puerperium and lactation of subject L.R.

DAYS OF PUERPERIUM	WEIGHT OF SUBJECT	NITROGEN INTAKE	NITROGEN OUTGO					NITROGEN BALANCE
			Urine	Feces	Pads	Breast milk	Irrigation	
1		15.67	13.93	2.75	0.87	....	0.08	— 1.96
2		14.38	12.70	....	7.95[1]	....	0.29	— 6.56
3		16.56	12.46	....	0.19	1.67	0.14	+ 2.10
4		18.52	13.11	3.95	0.53	5.17	0.38	— 4.62
5		16.10	11.32	4.46	0.24	5.17	0.17	— 5.26
6		17.74	16.76	1.39	0.22	5.87	0.50	— 7.00
7		17.20	16.26	1.39	0.17	6.14	0.46	— 7.22
8		15.77	14.65	1.39	0.04	6.88	0.38	— 7.57
9		15.19	13.71	1.39	0.05	6.50	0.09	— 6.55
Total		147.13	124.90	16.72	10.26	37.40	2.49	— 44.64
Mean		16.35	13.88	1.86	1.14	4.16	0.28	— 4.96
DAYS OF POST PARTUM								
10–17	65.9	17.56	13.85	1.41	0.51	4.21		— 2.32
18–22		16.75	12.24	1.49	0.51	2.64		— 0.13
23–27		19.00	12.10	1.58	0.24	2.52		+ 2.55
28–32		18.55	13.37	3.30	0.18	5.10		— 3.40
33–37	66.0	18.06	11.48	1.79	0.11	5.23		— 0.55
38–42		19.52	12.82	2.18	....	4.65		— 0.13
43–47		17.07	13.02	1.82	....	5.01		— 2.78
48–53		19.60	12.59	2.62	....	3.90		+ 0.49
Total		802.83	561.49	87.80	9.28	182.83		— 38.31
Mean		18.36	12.76	2.00	0.21	4.16		— 0.87

[1] Clot of blood lost.

the involuting uterus leaving 288.4 gm. of nitrogen for use during the succeeding days of lactation and postlactation. Many more cases will have to be studied intensively and continuously before it is possible to determine the actual nitrogen requirements of the average reproductive cycle including the sum total of the physiological gains and losses.

Lactation. During the 43 days of lactation following the period of puerperium of 9 days there was a maternal loss of 38.3 gm. of nitrogen or an average of 0.87 gm. daily as shown in table 3 and figure 3. The body weight of L.R. at the fifteenth day postpartum was 65.9 kilos, a weight approximating her usual non-gravid state. Altogether then the puerperium and 6 weeks of lactation decreased the 'rest nitrogen' from 333 to 250 gm. The inability to extend the continuous metabolic studies further into lactation made it impossible to determine when this maternal body reached an equilibrium and whether it was actually left enriched at the termination of the reproductive cycle or whether the 'reserve material' of gestation was completely dissipated. Data previously reported (Hunscher, Donelson, Nims, Kenyon and Macy, '33) on the response of the present woman to analagous reproductive metabolic states when she was consuming similar nitrogen intakes at the fiftieth week of the second lactation showed that she was storing 5.7 gm. of nitrogen per day and, at the seventh, twenty-sixth, thirty-sixth and sixty-second weeks of the third lactation the daily nitrogen balances were — 0.84, + 0.87, + 1.45 and + 0.61 gm., respectively. Furthermore, at the eleventh and forty-sixth weeks postlactation of the latter cycle the balances were positive with a lowered consumption of nitrogen.

From the continuous metabolic studies of this case during a large part of a reproductive cycle and at intervals in two preceding cycles it is evident that losses occur especially during early lactation in some multiparae[6*] but the maternal body can attain equilibrium and even store nitrogen during heavy milk flow after the twenty-sixth week. This observation was borne out in two other women with high milk production records (Hunscher, Donelson, Nims, Kenyon and Macy, '33). If the large losses of nitrogen in early lactation are due in part to involutional processes[6, 7*] of the maternal organs (Harding and Montgomery, '27) and are therefore physiologic, and, are provided for by the 'rest material' accumulated

[6*] and [6, 7*] See footnotes 6 and 7 on page 590.

in pregnancy, it seems important to search out methods of providing the mother with an ample reserve to aid against vital losses at the time of delivery and during the period when milk flow is being established. Whether excessive losses can be reduced by larger intakes of nitrogen (i.e., more than 18.4 gm. of nitrogen or 115 gm. of protein) or through other dietary and hygienic means remains to be investigated by further continuous balance studies and verified in clinical practice.

Only a few data are available on the nutritive needs for human milk secretion (Hoobler, '17; Courtney, '23; Adair, '25; Kleiner, Tritsch and Graves, '28). Since the subject of this investigation secreted a large quantity of breast milk which was completely expressed manually at regular intervals in each 24-hour period and the chemical composition determined during the entire study it is possible to assess the protein requirement under her specific metabolic demands. The maternal maintenance requirement, the loss in the milk secreted and the incalculable metabolic cost for the mammary gland activity in the synthesis of a milk of known quantity and quality constitute the protein need. L.R. secreted an average of 26.5 gm. of protein in her milk from the tenth to the fifty-third day of lactation. If her protein maintenance requirement did not exceed the usual 70 gm. allowance for the adult and if the requirement for milk secretion equals the outgo of protein in the milk then her calculable requirement amounted to 96.5 gm. daily. She used, however, an average of 120 gm. of protein daily as shown by the loss of 5.4 gm. over and beyond the 115 gm. accounted for in her food intake when as little as 26.5 gm. of protein occurred in breast milk. It seems then that nitrogen was not only used or dissipated in the physiological readjustment to the establishment and maintenance of milk flow but in the actual milk synthesis. In addition, it is possible that in some cases other factors may be operating, such as an undernourished state due to either an inadequate food supply or insufficient utilization of the food taken prior to conception, an actual pathological state during

gestation, or an inability of the body to retain the 'rest material' of pregnancy on the one hand, or on the other hand, a drain placed upon the maternal body through frequent and intensive reproductive cycles as may be true in the present subject. These considerations illustrate some of the difficulties involved in the computation of actual and general dietary requirements for reproduction and point to the need for more skillful dietary and clinical means of safeguarding the maternal tissues from undue deprivation.

The continuous metabolic balance studies reported herein show variations in retentions which, perforce, are inherent in data secured from isolated intermittent observations. The continuous findings are more valid since they show not only the general trend of metabolism during the different phases of the reproductive cycle but also show the great fluctuations that may occur in short periods under apparently similar experimental conditions. These results are in accord with the previous data on women undergoing the reproductive cycle in which we have shown that "there is a marked variation in the storage of nitrogen in the same individual despite the maintenance of a fairly constant consumption of protein, where consecutive balances are determined. Such evidence indicates that a single balance does not represent a physiological constant of metabolism and consequently it should not be used too freely in generalizations" (Hunscher, Donelson, Nims, Kenyon and Macy, '33). Likewise, the present data are singularly substantiated through observations obtained in successive nitrogen balances in childhood.[8]

From the data accumulated in this laboratory over a period of years it appears that the most satisfactory means of assessing the physiologic load of maternity and of determining the nutritive state of women at the termination of the reproductive cycle is by observing continuously the retention or loss of the various elements throughout each successive phase

[8] An elaborate study on successive metabolic balances in childhood is to be published at an early date (Hunscher, Cope, Noll, Macy, Cooley, Penberthy and Armstrong, '32; Hunscher, Cope, Noll and Macy, '33).

of pregnancy, delivery, puerperium and lactation with a consequent accounting of the total demands made upon the maternal tissues throughout the entire cycle. It has been a rare and unprecedented opportunity to be able to collect from the same individual during the major portion of her years of child-bearing and child-rearing metabolic data from three successive and physiologically similar reproductive cycles covering almost 8 years and to be able to supplement them with observations on women satisfactorily carrying similar metabolic loads. In addition, it is a matter of practical health interest to learn the consequential effect of child-bearing upon the well-being of the mother and her child under various types of normal conditions and abnormal states that frequently arise in pregnancy, parturition, puerperium and lactation.

It is through the accumulation of many varieties of chemical exchange data on numerous women beginning, if possible, before conception and following throughout pregnancy, parturition, puerperium and lactation and even beyond, until the maternal body reaches a state of equilibrium that knowledge for the solution of so many urgent problems will be found. With a fuller knowledge of the maternal metabolic processes, physicians will be better prepared to guide mothers to enjoy buoyant health and at the same time to bear nutritionally stable children.

SUMMARY

A case study extending over an 8-year period of child-bearing and child-rearing has been made on a woman when she and her children were known to enjoy buoyant health.

Uninterrupted nitrogen metabolic responses during the last half of fetal development and the physiological preparation of the maternal body for lactation and the extension of these observations into parturition, puerperium and 8 weeks of lactation showed where some of the stresses and strains of maternity lay. The results confirm previous considerations derived from intermittent balances in two former reproductive cycles in the same woman.

During the last 145 days in gestation there was an average net storage of 3.1 gm. and a maternal retention of 2.6 gm. of nitrogen daily resulting in a total observed accumulation of 446 gm. at term.

On the day of delivery the chemically determined maternal loss in blood, placenta, amniotic fluid and vomitus amounted to 46.0, 20.1, 0.08, 0.24 gm. of nitrogen, respectively; the total loss from the body beyond the food consumed amounted to 54.6 gm. of nitrogen in addition to that contained in the fetus.

The nine daily balances during the lying-in period showed an average daily loss of 5 gm. of nitrogen.

From the tenth to the fifty-third day of lactation there was an average daily loss of 0.87 gm. of nitrogen.

By the fifty-third day of milk flow the gestatory reserve nitrogen had been reduced by delivery, puerperium and lactation losses of 54.6, 44.6 and 38.3, respectively leaving a total of 310 gm. of nitrogen stored only in the last half of pregnancy. When the approximate fetal content of 58.6 gm. of nitrogen is deducted from the final maternal reserve, the accountable losses of the reproductive cycle by the fifty-third day of lactation had left a maternal reserve of 250 gm. of nitrogen for future dissipation or enrichment of the maternal body at termination of the reproductive cycle.

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SELENIUM IN PROTEINS FROM TOXIC FOODSTUFFS [1]

IV. THE EFFECT OF FEEDING TOXIC PROTEINS, TOXIC PROTEIN HYDROLYSATES, AND TOXIC PROTEIN HYDROLYSATES FROM WHICH THE SELENIUM HAS BEEN REMOVED

KURT W. FRANKE AND E. PAGE PAINTER

Department of Experiment Station Chemistry, South Dakota State College, Brookings

TWO FIGURES

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It was stated in an earlier paper (Franke and Painter, '35 a) that the only difference known between 'normal' and toxic foodstuffs is the presence of selenium in the latter. Vanadium may contribute to the toxicity of some of the grains as Byers ('34) found this element in one sample. Beath, Eppson and Gilbert ('35) have reported that plants may take up molybdenum in amounts which will produce toxic symptoms when fed to livestock. They state that molybdenum is less toxic than selenium.

Franke ('34 b) reported that the protein carried the toxicant in toxic foodstuffs and Robinson ('33) and Franke and Painter ('35 a) found the selenium in the protein. The latter workers also reported that the selenium was in organic combination in the protein and was in solution after the protein was hydrolized. Painter and Franke ('35 b) have developed

[1] Published with the permission of the director of South Dakota Agricultural Experiment Station as communication no. 17 from the department of experiment station chemistry. These investigations are being carried out under the Purnell Fund and with the cooperation of the Bureau of Chemistry and Soils, Bureau of Plant Industry, Bureau of Animal Industry, and Bureau of Home Economics of the United States Department of Agriculture.

a procedure whereby the selenium compounds can be removed from the hydrolysate.

Several papers have appeared from this laboratory on the effects of toxic foodstuffs on the animal body. However, no report has appeared on the toxicity of the derived products of toxic proteins.

In view of the above facts, it seemed advisable to determine by growth studies on the albino rat the relative toxicity of the grain, the protein, and the protein hydrolysate, and to determine if the removal of selenium would render the protein hydrolysate non-toxic.

EXPERIMENTAL

In order to determine the effect on animals of the chemical treatment used to separate the selenium compounds, the following feeding trials (series 101) were carried out.

Group A was fed a diet containing toxic wheat.

Group B was fed a diet containing toxic proteins.

Group C was fed a diet containing the hydrolysate from toxic proteins.

Group D was fed a diet containing the hydrolysate from toxic protein from which the selenium had been removed.

It should be emphasized that in these feeding trials strict series of controls were used. In all feeding trials a fraction was fed from a 'normal' wheat (laboratory no. 648) which had received the same treatment as the experimental. These will be referred to as control groups A, B, C and D.

The protein from toxic wheat (laboratory no. 582) fed to group B was prepared by the same procedure as that used in previous investigations (Franke and Painter, '35 a).

The following method was used to prepare the hydrolysates for animal feeding. The hydrolysis of proteins has been described (Painter and Franke, '35 a). The insoluble humin was filtered off and the sulfuric acid removed in the manner already described for preparing the hydrolysates prior to the separation procedures (Painter and Franke, '35 b). Care had to be taken to ensure that there was no barium in the

hydrolysate. It was found that excess sulfuric acid had to be present before all the barium was removed. The pH of the hydrolysate was acid (between 4 and 5), but indicators could not be depended upon. Instead dilute sulfuric acid was added until no more barium sulfate precipitated, then several cubic centimeters of very dilute acid were added. The solution was concentrated to a thick syrup by vacuum distillation at not over 60°C. The syrup was removed from the distillation flasks and placed in evaporating dishes. At this time sodium hydroxide was added until the end point of methyl red was reached. The neutral hydrolysates were then placed on a steam bath to remove most of the water, then finally dried in a vacuum oven at 60°C. The amino acids were then ground in a mortar, and kept in a desiccator until incorporated into diets and fed to group C.

Preparation of the selenium free hydrolysates

The method of precipitating the selenium from toxic protein hydrolysates has been described (Painter and Franke, '35 b), but the essential features of the precipitation will be restated.

The neutral hydrolysate (free from sulfates and barium) was diluted to a volume of 30 cc. for every gram of protein hydrolized. Solid barium carbonate was added in excess, then for every 30 cc. of hydrolysate 20 cc. of saturated mercuric chloride was added while the solution was stirred. The solution was allowed to set at room temperature, with frequent stirring, for 1 hour. The flaky mercury precipitate and undissolved barium carbonate was filtered off, using a Buchner funnel, and the precipitate washed several times with water. A precipitate may form in the clear solution after the mercuric precipitate of amino acids and excess barium carbonate have been filtered off if it is allowed to stand, but this may be disregarded. The mercury in the filtrate was removed with hydrogen sulfide and the barium with dilute sulfuric acid. The mercury, barium and sulfate-free filtrate was evaporated to near dryness several times in vacuum to remove as much chlorine as possible. The filtrate was then made up to a

suitable volume and the remaining chlorine removed by the addition of boiling hot suspensions of silver oxide. This was added until the pH reached 4. The silver chloride and excess silver oxide was then filtered off and washed several times with hot water. The small amount of silver in the solution was removed as the sulfide by the addition of hydrogen sulfide and the volume of the solution reduced by vacuum distillation until some amino acids began to crystallize out. The solution was then tested with a few cubic centimeters of dilute sulfuric acid to ensure complete removal of barium. If it was free from all toxic metals, dilute sodium hydroxide was added and the pH adjusted to that described for the hydrolysates. This amino acid fraction was then dried in the manner already described. Each one of these filtrates was tested for the presence of selenium before it was incorporated into diets and fed to group D. The same procedure was carried out using a hydrolysate of control protein which served as a control group (D). The usual practice was to hydrolize 300 gm. of protein at one time and divide the neutral hydrolysate, taking half for the hydrolysate diet and carrying the other half through the precipitation procedure.

Preparation of the diets

The diets were prepared so that their nitrogen contents were equal. It has been stated (Franke, '34 b; Franke and Painter, '35 a) that the toxicant was carried in the protein of toxic foodstuffs so protein nitrogen was used as the basis for the preparation of the diets.

The wheat diets were the same as those used by Franke and Potter ('34). The following table gives the diets used in each of the eight groups:

Toxic and control groups	SERIES 101 DIETS			
	A	B	C	D
Component in per cent				
Ground whole wheat	82			
Commercial casein	10	10	10	10
Cod liver oil	2	2	2	2
Dehydrated yeast (Northwestern)	2	2	2	2
McCollum's salt mixture no. 185	1	3	2	2
Lard	3	5	5	5
Cornstarch		15	15	15
Commercial sugar		45.2[1]	43.2[2]	41.8[2]
Protein		17.8[1]		
Protein hydrolysates			20.8[2]	
Protein hydrolysates after $HgCl_2$ pptn.				22.2[2]
Cystine (E. & A., C.P.)			00.1	00.1

[1] This was not the same for the control and experimental diet because the proteins used did not contain the same percentage of nitrogen.

[2] The different diets varied as the nitrogen content of the hydrolysates. The figure given represents an average figure, but not the percentage in every diet. The material was being prepared while the feeding was in progress so the weight of the hydrolysates and sugar varied somewhat depending upon the percentage of nitrogen in the hydrolysates.

Fourteen and five-tenths (14.5) per cent of the protein (N $\times$ 6.25 $\times$ 100) in the toxic wheat diet is in the whole wheat added. All other diets, except the control whole wheat diet, contained the same amount of protein nitrogen as this diet. This difference in the nitrogen in the protein and hydrolysates necessitated varying another constituent of the diet. The sugar was the constituent varied.

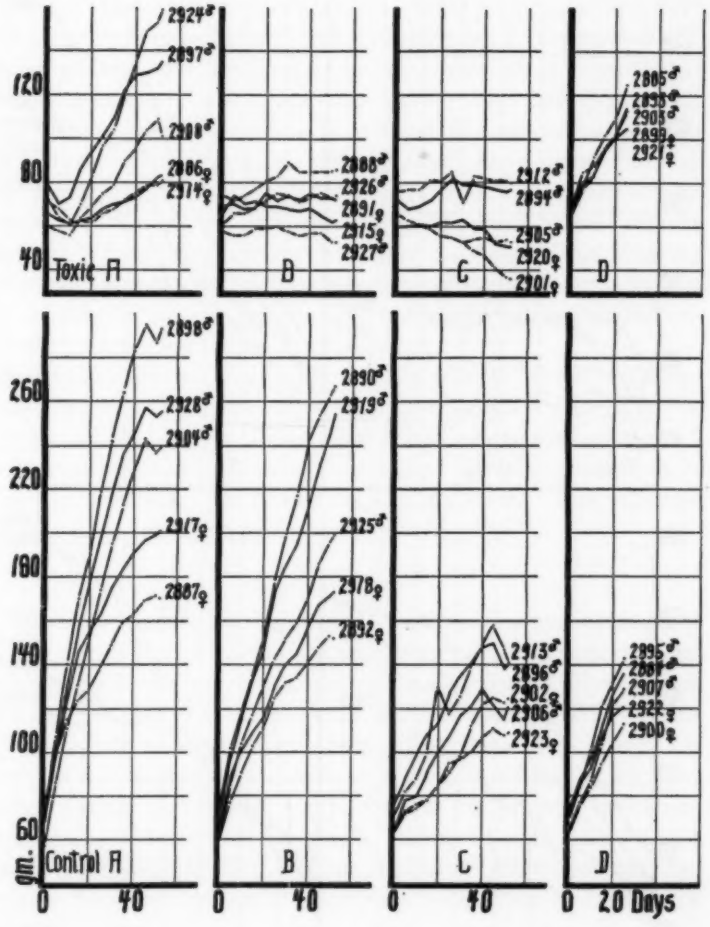
The average nitrogen content for the hydrolysates was approximately 11.2 per cent and for the amino acids resulting from mercuric chloride precipitation 10.4 per cent. It is realized that this is somewhat low; however, the weight of a protein hydrolysate increases over that of the protein, some of the ammonia nitrogen may have been lost and the hydrolysates were contaminated with small amounts of salt. The nitrogen content of the toxic protein was 13.0 per cent and of the control protein was 13.8 per cent. These figures seem to be very low, indicating that a pure protein was not used. Mitchell and Hamilton ('29) give 17.66 and 17.49 per cent,

respectively, for gliadin and glutenin, the chief proteins in wheat gluten. The proteins were purified by the method of Sandstedt, and Blish ('33). Both were taken from wheats of high protein content, 17.66 per cent for the toxic (582) wheat, and 17.5 per cent for the 'normal' (648) wheat. The factor 6.25 was used to convert nitrogen to protein. It appears that the method of Sandstedt and Blish did not separate all the protein from the starch. Further purification by dispersing the protein in alkali did not seem advisable because it has already been shown that the selenium in toxic proteins is unstable in alkaline solution.

Albino rats of Wistar Institute origin were the experimental animals used. They had been weaned on the twenty-first day and maintained on McCollum's diet I (Burr and Burr, '29) for 7 days. They were then divided into eight groups so that litter mates and the same number of males and females were in each group, and placed in individual cages described by Franke and Franke ('34). The average weight of the eight groups did not vary 0.5 gm. from the mean. The rats were weighed every 5 days and the food consumption recorded every 2 days.

Individual growth curves for the eight groups of series 101 are shown on figure 1.

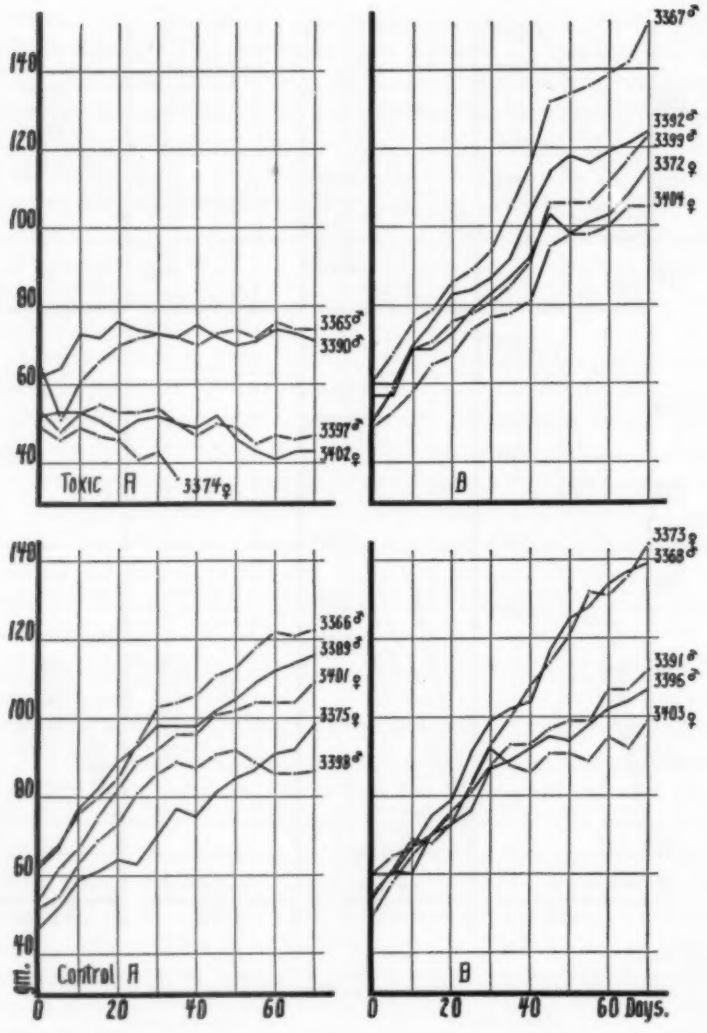
Due to the results obtained in the toxic and control groups C and D it seemed advisable to duplicate these four groups. The toxic hydrolysate which had the selenium removed appeared to be non-toxic; however, it was felt that a period of 25 days was not long enough to warrant conclusions. Furthermore, better growth was obtained in control group D, which had part of the amino acids removed by mercuric chloride precipitation, than in control group C. Also accurate food consumptions were not obtained during the first 20 days of series 101 on the groups receiving amino acid diets. This difficulty was overcome by adding water to the diet and making it into a thick soup when it was fed. Rose (personal communication) obviates this difficulty when feeding amino acid diets by using diets with a high fat content.



P-1 S-101

Fig. 1 Individual growth curves for series 101.

(Toxic) groups		*(Control) groups*
A	Whole wheat	A
B	Protein	B
C	Hydrolysate	C
D	Hydrolysate after $HgCl_2$ precipitation	D



P-2 S-112.

Fig. 2 Individual growth curves for series 112.

(*Toxic*) *groups*		(*Control*) *groups*
A	Hydrolysate	A
B	Hydrolysate after $HgCl_2$ precipitation	B

Series 112 was therefore planned. Four groups of rats were fed diets similar to those given groups C and D in series 101. These groups are designated as toxic groups A and B and control groups A and B (series 112). The following alterations of the method used in preparing the amino acids were made, otherwise the same experimental procedure was followed. The hydrolysates were neutralized with solid sodium carbonate instead of sodium hydroxide, and silver sulfate prepared in this laboratory was used to remove the chlorine from the filtrate after the mercuric chloride precipitation. The sulfate was then removed with barium hydroxide following the precautions already mentioned.

Individual growth curves for these groups which extended to the seventieth day are shown in figure 2.

All animals in both series were killed at the end of the experimental period and autopsies made. In some cases hemoglobin determinations were made following the method already described by Franke and Potter ('34).

DISCUSSION

The three control groups A, B and C of series 101 show clearly the nutritional values of synthetic and natural diets. The poorer growth rate using the protein and hydrolysate diet is clearly shown. The depression of growth after the fortieth day in the group fed the control hydrolysate diet (group C) may have been due to a slight amount of barium in the diet. It was found that sulfuric acid had to be present in excess to completely remove the barium. Rapport and Beard ('27) observed difficulty with barium toxicity when barium hydroxide was used to remove sulfuric acid from hydrolysates used in animal feeding.

There were no deaths in series 101 although this grain sample (laboratory no. 582) is one of the lethal samples reported by Franke ('34 a) in which over 70 per cent of the animals died before the sixtieth day of experimentation. This may have been due to the fact that the animals averaged 2 to 3 days older when placed on experiment than those reported by Franke. It has been shown (unpublished data)

that older animals have a greater resistance to the toxicity of selenium containing grains.

If depression of growth may be taken as a measure of toxicity it is quite evident that the protein is just as toxic after it has been separated from the wheat, dispersed in acetic acid and re-precipitated. The results here indicate that the protein diet is more toxic; however, it should be observed that the growth of the control group B is not as good as that in the control group A. Other feeding trials using toxic proteins indicate that the toxicity is not reduced by the method employed in its separation.

It is difficult to state whether or not the hydrolysate diet (group C) is as toxic as the whole wheat and protein diets. Growth was profoundly suppressed, but the control group also showed marked growth depression when compared with the groups receiving control wheat and control protein diets. The results suggest that the toxicity of the hydrolysate, due to the toxicant in the original grain, had been reduced.

The alkaloidal test indicated that the selenium contents of the toxic diets fed to groups A, B, C (series 101) and toxic group A (series 112) were the same. The method described by Robinson et al. ('34) gives 31 p.p.m. of selenium for the whole grain, 120 p.p.m. of selenium for the toxic protein, and 100 p.p.m. of selenium in the toxic hydrolysate. If these figures are correct, there is more selenium in the toxic grain diets than in the toxic protein or hydrolysate diets. The protein diets contain more selenium than the hydrolysate diets but the difference is hardly significant. It has been shown (Painter and Franke, '35 a) that considerable selenium is in the insoluble humin when toxic proteins are hydrolized with acids.

In both series better growth resulted in the control groups with the diet containing the hydrolysate after part of the amino acids had been removed by mercuric chloride precipitation than in the groups whose diet contained the untreated hydrolysate. The obvious question is: Did the mercuric chloride precipitation remove some growth depressant or toxicant present in protein hydrolysates? Or were the results

due to the removal of something rendering the diet more palatable? The change in color of the hydrolysate after the mercuric chloride precipitation indicated that much of the soluble humin had been removed. Undoubtedly the gustatory effect of protein hydrolysates in diets is a contributing factor in the failure of animals to respond favorably to these diets.

The necropsy of the animals revealed the usual symptoms produced by toxic foodstuffs. The severe pathological disturbances (Franke, '34 a) were not as acute in the animals receiving a toxic protein hydrolysate diet as in the animals receiving the toxic wheat and toxic protein diets. The external appearance of the animals in toxic group C (series 101) and toxic group A (series 112) was typical of that generally produced by toxic foodstuffs. The animals on toxic diets were very thin and in general appeared emaciated. All groups receiving control foodstuffs appeared normal except that those receiving the diets containing amino acids were much smaller. This was also true of the animals receiving toxic protein hydrolysates from which the selenium had been removed. They did not exhibit the typical pathological disturbances observed in the other groups.

Several hemoglobin determinations did not reveal any abnormally low levels in rats fed toxic protein hydrolysates which Franke and Potter ('35) observed in rats fed toxic grain. Only a few samples of blood were taken when the animals were killed and these were not from moribund animals. The two lowest levels obtained in series 112 were rats no. 3397 and no. 3402, which had hemoglobin levels of 14 and 12.6 gm. per 100 cc. blood, respectively. Those animals in the control group and protein hydrolysate with selenium removed had normal levels.

The voluntary restriction of food consumption observed (Franke, '34 a) in animals fed toxic grain diets was observed in the animals fed toxic protein hydrolysate diets. In series 112 there is a direct correlation between growth and food consumption. The available records of series 101 indicate that the growth rates and food consumption were in direct proportion. The average food consumption in grams per day

for the toxic groups A and B (series 112) was 3.32 and 6.33 and for the control groups A and B was 5.49 and 6.39. It is evident that the restriction of food consumption is due to the toxicant present in the whole wheat, protein and hydrolysate diets.

Both series 101 and 112 prove without question that the removal of selenium by the method described removes the toxicant present in toxic protein hydrolysates. This is clearly shown by comparing toxic groups C and D of series 101 and toxic groups A and B of series 112. The growth rate, external appearance of the animals, symptoms observed at autopsy and response to the diets all indicate that the toxicant was removed by the mercuric chloride precipitation so completely that the amount left was innocuous. Either selenium in some compound (or compounds) is the sole toxicant or other toxicants, if present, are likewise precipitated by mercuric chloride. The results obtained do not prove that selenium is the sole toxicant present in toxic foodstuffs but since no toxicant was removed from either toxic proteins or their hydrolysates by organic solvents, nearly all known plant toxicants are eliminated. Observations feeding selenium salts (Franke and Potter, '35) indicated that their action on the animal body is very similar to that of toxic grains, although the symptoms produced were not exactly or consistently the same.

SUMMARY AND CONCLUSIONS

The effect of chemical treatment of the proteins from toxic wheat has been studied by animal feeding. Growth curves of animals fed diets containing whole grain, protein, protein hydrolysate and protein hydrolysate after mercuric chloride precipitation are shown.

From the results obtained by the separation trials and feeding series, the following conclusions are made:

1. The sulfuric acid hydrolysates of toxic proteins are toxic.

2. Mercuric chloride precipitation of toxic protein hydrolysates (under the proper conditions) precipitates the selenium compound (or compounds) so nearly completely that the filtrate is innocuous when fed to albino rats.

3. Mercuric chloride precipitation removes something from sulfuric acid hydrolysates which directly or indirectly inhibits growth.

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THE RELATION BETWEEN THE VITAMIN A AND D INTAKE BY THE HEN AND THE OUTPUT IN EGGS [1]

WALTER C. RUSSELL AND M. W. TAYLOR

Department of Agricultural Biochemistry, New Jersey Agricultural Experiment Station, and Rutgers University, New Brunswick

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Early in the history of vitamin studies McCollum and Davis ('13) and Osborne and Mendel ('14) showed that egg yolk contained a growth-promoting substance which later came to be known as vitamin A. Probably the earliest demonstration of the influence of the vitamin A content of the ration on the amount of this factor in the egg was by Bethke, Kennard and Sassaman ('27). Mellanby ('21) and later Hess ('23) reported that egg yolk possessed antirachitic properties. That the antirachitic potency of egg yolk could be increased by exposure of the hen to ultraviolet light was shown almost simultaneously by Hart, Steenbock and associates ('25) and by Hughes, Titus and Moore ('25). Bethke and coworkers ('27) confirmed this finding and added the observation that the antirachitic potency of egg yolk could be influenced by the amount of the factor in the ration.

The principal objective of this investigation was the determination of any relationship between the vitamin A and D intake and the quantity of these factors which appears in the eggs.

[1] Journal series paper of the New Jersey Agricultural Experiment Station, Department of Agricultural Biochemistry. Presented before the Division of Biological Chemistry of the American Chemical Society in Cleveland, Ohio, September, 1934.

EXPERIMENTAL

Feeding plan. Three groups of four hens each were housed in individual compartments of a fattening battery on December 18, 1933. They were just entering their first laying period and during the preceding summer had been on range until August 1st, after which time they were in open front houses. The cages were equipped with specially constructed feeding cups which permitted accurate measurement of feed consumption. Mash feed, table 1, was available at all times but the scratch grain was given daily in weighed amounts. Although the original plan was to control the supply of scratch grain so that equal amounts of mash and scratch would be consumed daily, on account of the lack of exercise it was considered advisable to limit the allowance of scratch grain to approximately one-half the ad libitum mash consumption. Grit and oyster shell were fed separately.

Vitamin A and D assays. The vitamin A content of the cod liver oil, yellow corn, and egg yolk was determined essentially by the Sherman and Munsell ('25) method. Since the completion of this work, there has come into use the U. S. P. X 1934 revision (Interim Revision Announcement, '34) of the method for the assay for vitamin A in cod liver oil. This method is the same in principle as the procedure described by Sherman and Munsell except that a 4- instead of an 8-week assay period is used and a reference oil whose potency has been agreed upon is employed as a standard. Since a number of laboratories are using this reference oil, results comparable with those of other workers can best be obtained by referring the rat growth response at 4 weeks to a dosage-response curve for the reference oil and expressing the potency in terms of U. S. P. X 134 units. Unless otherwise stated the U. S. P. X 1934 unit is used in this publication.

Carotene was determined colorimetrically by the method developed originally by Willstätter and Stoll ('13) and modified by Russell, Taylor and Chichester ('35).

The value in biological units assigned to alfalfa meal was based upon its carotene content, at least a rough correlation

having been shown to prevail between the carotene content and the vitamin A value determined biologically (Russell, Taylor and Chichester, '34). It is recognized that the vitamin A potency of alfalfa meal and yellow corn, particularly if the latter is ground, will decrease during storage, but the values assigned to corn and alfalfa meal are considered to be typical of those products.

The egg yolk was fed suspended in a 1 per cent sodium chloride solution as a separate supplement, 1 to 2 cc. of the suspension being the volume which contained 1 dose. It was found convenient to draw the egg yolk into a Luer syringe in which condition it could be preserved in the refrigerator with little chance of spoilage because the yolk at the tip of the syringe hardened to a self seal. Eggs collected from the groups of four hens each during February were not pooled but were broken as required for the preparation of supplements, the number of eggs used from each hen being proportional to the number produced. Likewise eggs were broken as required for the assay in the case of individual hens.

The vitamin D potency of the cod liver oil and egg yolks was determined by the line test method essentially as originally described by McCollum, Simmonds, Shipley and Park ('22), and more recently used by the Wisconsin Alumni Research Foundation, and expressed in Steenbock units. The substances under examination were admixed with the ration. After the completion of this work, the U. S. P. X 1934 revision of the method for the assay for vitamin D in cod liver oil came into use. This method is essentially the same as that of the Wisconsin Alumni Research Foundation but it involves the use of a reference oil whose potency is expressed in U. S. P. X 1934 units and which has been agreed upon as a standard by a number of laboratories. In order to convert Steenbock units to U. S. P. X 1934 units the factor 3.2 was used, 1 Steenbock unit being equivalent to 3.2 U. S. P. X 1934 units for this animal colony.

RESULTS AND DISCUSSION

Vitamin A studies

The rations, table 1, were designed for the study of the effect of an increase in vitamin A concentration of the ration on egg production, potency of egg yolk, and percentage output of the factor in the eggs. The concentration of vitamin A in the ration could have been markedly increased by the addition

TABLE 1

Composition of rations

	VARIABLE CONSTITUENTS OF THE MASH		
	Group 1	Group 3	Group 4
	per cent	*per cent*	*per cent*
Yellow corn	0.0	32.0	27.0
White corn	32.0	0.0	0.0
Alfalfa meal	0.0	0.0	5.0

Constant constituents of the mash

Wheat bran, 18.5 per cent; wheat middlings, 18.5 per cent; pulverized oats, 9.0 per cent; meat scrap, 18.0 per cent; oyster shell, ground, 3.0 per cent, and salt (NaCl), 1.0 per cent. One part of cod liver oil no. 2543 added per 100 parts of mash.

	SCRATCH GRAIN		
	Group 1	Group 3	Group 4
	per cent	*per cent*	*per cent*
Yellow corn, cracked	0	32	50
White corn, cracked	50	18	0
Whole wheat	50	50	50

One part of cod liver oil no. 2543 added per 100 parts of scratch.

of carotene but it was desired to keep the vitamin A potency within the limits which are possible with the use of practical and commonly used feeds. The vitamin A potency of the constituents of the ration carrying this factor and of the egg yolks are displayed in table 2. The weight of an egg yolk, 16 gm., was adopted after the weighing of a number of the yolks used in the experiment. The percentage output of the factor in the eggs expresses the relation between intake and

output, but does not take into consideration the possibility that some of the vitamin A in the eggs may have come from the body stores.

The number of individuals under observation, table 3, was small and it is recognized that marked differences in performance of the individuals of a group of hens on the same

TABLE 2

Vitamin A content of rations and eggs

CONSTITUENT	VITAMIN A PER GRAM U. S. P. X 1934 UNITS
Individual constituents	
Cod liver oil no. 2543	1400
Yellow corn	7
Alfalfa meal	120
Rations	
Group 1	
Mash	1400
Scratch grain	1400
Group 3	
Mash	1625
Scratch grain	1625
Group 4	
Mash	2200
Scratch grain	1750

Eggs

FEBRUARY, 1934	VITAMIN A PER 16 GM. YOLK U. S. P. X 1934 UNITS	MAY, 1934	VITAMIN A PER 16 GM. YOLK U. S. P. X 1934 UNITS
Group 1	540	Group 1, hen no. 100	600
Group 3	500	Group 3, hen no. 54	800
Group 4	540	Group 4, hen no. 48	680

ration are always found. However the results permit the calculation of the amount of vitamin A in the eggs as a percentage of that consumed.

The number of units of the factor ingested by group 4 in February was almost twice that by group 1 and the number of eggs produced by group 4 was of the order of twice that

by the latter group. Since the vitamin A potency of the eggs was the same for these two groups, the percentage of the vitamin in the ration which appeared in the eggs was essentially the same for both groups, 16 and 17 per cent, respectively. The individual hens studied in May, table 4, consumed more ration and consequently more vitamin A than they did in February and produced more eggs. Also the vitamin A potency of the yolks of eggs from individual hens was higher in May than the values obtained for the pooled eggs in

TABLE 3

Vitamin A intake in the feed and output in the eggs. February, 1934. U. S. P. X 1934 units

HEN NO.	FEED INTAKE		EGGS LAID	VITAMIN A INTAKE			VITAMIN A OUTPUT IN EGGS	VITAMIN A OF RATION WHICH APPEARED IN EGGS
	Mash	Scratch		Mash	Scratch	Total		
				Group 1				
	gm.	*gm.*		*units*	*units*	*units*	*units*	*per cent*[1]
27	1225	650	5	17,150	9,100	26,250	2,500	10
61	1280	650	12	17,920	9,100	27,020	6,000	22
67	520	650	2	7,280	9,100	16,380	1,000	6
100	1165	500	10	16,300	7,000	23,300	5,000	21
Total	4190	2450	29	58,650	34,300	92,950	14,500	Average 16
				Group 3				
10	890	650	0	15,460	10,560	25,020	0	0
17	1090	650	4	17,720	10,560	28,280	2,160	8
24	860	650	6	13,990	10,560	24,550	3,240	13
54	1765	650	8	28,700	10,560	39,260	4,320	11
Total	4605	2600	..	74,870	42,240	117,110		
Total for laying hens	3715	1950	18	60,410	31,680	92,090	9,720	Average 11
				Group 4				
46	1610	650	17	33,320	11,380	44,700	9,180	21
49	820	650	6	18,050	11,380	29,430	3,240	11
74	1790	650	20	39,400	11,380	50,780	10,800	21
96	1185	650	8	26,100	11,380	37,480	4,320	11
Total	5305	2600	51	116,870	45,500	162,390	27,540	Average 17

[1] Percentages are given to nearest whole number.

February. The net result of the changes in vitamin A consumption, egg production and possible increase of egg yolk potency (in the case of individual birds), was an increase in the percentage of vitamin A of the ration which appeared in the eggs in May. The percentage values were 32 and 27 per cent, respectively, for groups 1 and 4 for May as compared with 16 and 17 per cent in February. Group 3 was not producing as well as nos. 1 and 4 in February and the percentage output of the factor in the eggs was low, 11 per cent, although the average consumption of vitamin A per hen was greater than that of group 1. In May the representative of group 3 showed a percentage output of the vitamin of the same order

TABLE 4

Vitamin A intake in the feed and output in the eggs by individual hens. May 1934. U. S. P. X 1934 units

GROUP NO.	HEN NO.	FEED INTAKE		EGGS LAID	VITAMIN A INTAKE			VITAMIN A OUTPUT IN EGGS	VITAMIN A OF RATION WHICH APPEARED IN EGGS
		Mash	Scratch		Mash	Scratch	Total		
		gm.	*gm.*		*units*	*units*	*units*	*units*	*per cent*
1	100	2745	620	25	38,500	3,700	47,200	15,000	32
3	54	2960	620	22	48,200	10,100	58,300	17,600	30
4	46	1675	620	19	36,900	10,850	47,750	12,920	27

as that of the other groups. These percentages are of the same order as a number of those reported by Sherwood and Fraps ('32), although in a few cases they reported higher values.

At the end of the experiment the vitamin A potencies of the livers of the eleven surviving birds were determined by the antimony trichloride method according to Moore ('30) and the values expressed as blue units (Moore) per gram (table 5). When the blue unit values are arranged according to the decreasing order of the number of eggs laid by individuals during the 5-month period, they are found to fall, with one exception, hen 67, pen 1, in the reverse order. Thus the best producers had the lowest A potency per gram of liver in a given pen. Presumably the highest producers were drawing

more heavily on their vitamin A stores than the lower producers and thus would end a laying season with a low vitamin A store in the body.

Vitamin D studies

In an earlier experiment, 1932–1933 (unpublished data), hens were fed a ration similar to that employed in this experiment but they were kept in an open front house and 2 per

TABLE 5

Relation between the vitamin A in the liver and egg production

HEN NO.	EGGS LAID IN 5 MONTHS	BLUE UNITS (MOORE) PER GRAM OF LIVER
Group 1		
100	84	24
61	61	Neg.
27	16	435
67	14	350
Group 3		
54	69	107
17	48	121
24	43	182
10	13	332
Group 4		
74	102	42
46	92	88
96	27	200

cent of the same cod liver oil employed in the present experiment was incorporated in the mash. In January, 1933, the eggs from these birds contained 16 U. S. P. X 1934 units of vitamin D per yolk, table 6; essentially the same potency that was found in February in the present experiment. The potency of egg yolk in May, 1933, was 32 units per egg whereas in June, 1934, the number of units per yolk was less than 13, probably of the order of 6. The difference between the potencies observed in May, 1933, and June, 1934, was undoubtedly due to the sunlight received by the hens in the open front

house as compared with the lack of sunlight when the hens were caged indoors in the present experiment. The data suggest that sunlight is more effective in increasing the antirachitic potency of egg yolk than the amount of cod liver oil ordinarily used in the case of the hen; a suggestion which is not in agreement with the conclusions of Branion, Drake and Tisdall ('35).

TABLE 6

Vitamin D intake in the feed and output in the eggs. U. S. P. X 1934 units

	VITAMIN D PER 16 GM. YOLK
January, 1933	16
May, 1933	32
February, 1934	13
June, 1934	Less than 13

Group 4, February, 1934

HEN NO.	FEED INTAKE		EGGS LAID	VITAMIN D INTAKE			VITAMIN D OUTPUT IN EGGS	
	Mash	Scratch		Mash	Scratch	Total		
	gm.	*gm.*		*units*	*units*	*units*	*units*	*per cent*[1]
46	1510	650	17	1220	530	1750	218	12
49	820	650	6	660	530	1190	77	6
74	1790	650	20	1440	530	1970	256	13
96	1185	650	8	950	530	1480	103	7
Total	5305	2600	51	4270	2120	6390	654	Average 10

Vitamin D in the ration—the mash and scratch grain each contained 80 units of vitamin D per 100 gm.

[1] Percentages are given to nearest whole number.

As shown in table 6, 10 per cent of the vitamin D consumed appeared in the eggs in the case of group 4. The values for the antirachitic potency of egg yolk (on a comparable unit basis) are somewhat lower than those reported by Branion and associates ('35).

Experiments in this and other laboratories have shown that less than 4 per cent of the vitamin A (Baumann, Steenbock, Beeson and Rupel, '34, and Russell, Taylor, Chichester and Wilson, '35) and less than 2 per cent of the vitamin D (Russell,

Wilcox, Waddell and Wilson, '34 and Krauss, Bethke and Wilder, '34) of the cow's ration appear in the milk when adequate amounts of these factors are fed. In the case of the hen, 11 to 32 per cent of the vitamin A and 10 per cent of the vitamin D of the ration were found in the eggs. Hence the latter species is more efficient as a transmitter of these two vitamins. The fact that the fat content of the edible portion of eggs is 10 per cent as compared with 3.5 to 4.0 per cent for milk may be the explanation of the difference, because both of these vitamins are fat soluble and might be expected to be present in larger amount in the medium containing the higher percentage of fat.

We are indebted to Dr. D. F. Chichester of this laboratory for the determination of the blue units of vitamin A in the livers of the birds.

SUMMARY

The output of vitamin A in eggs, calculated as the percentage of that consumed, varied from 11 to 32 per cent, and was determined by the number of units of the factor consumed, the number of eggs produced and the potency of the yolk. The highest percentages were obtained during high production.

The liver vitamin A of the hens varied inversely with egg production.

In the case of vitamin D the amount of the factor which appeared in the eggs was 10 per cent of that consumed.

The data suggest that sunlight is more effective in increasing the antirachitic potency of egg yolk than the amount of cod liver oil ordinarily fed.

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A STUDY OF THE NUTRITIVE VALUE OF MUSHROOMS [1]

F. W. QUACKENBUSH, W. H. PETERSON AND H. STEENBOCK
Department of Agricultural Chemistry, University of Wisconsin, Madison

FIVE FIGURES

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INTRODUCTION

The nutritional value of the mushroom has long been a subject of speculation. Although most of the interest has been directed to the protein fraction, little can as yet be said with certainty regarding the actual proteins present and their digestive and nutritive properties. Less extensive studies by Orton, McCollum and Simmonds ('22), Hara ('23), Steidle ('24), and Scheunert and Reschke ('31) on the vitamins in mushrooms have revealed the presence of vitamin A activity in certain varieties, conflicting evidence on the vitamin B content, and little or no vitamin C. Sumi ('33) has found a considerable amount of ergosterol as the precursor of a vitamin D, and Scheunert and Reschke ('31) a vitamin D itself. In a comparison of the nutritional value of mushrooms with that of vegetables, Sabalitschka ('31) has reviewed the occurrence of various other dietary constituents of mushrooms, such as fat, sugar, nitrogen-free extract, fiber and ash.

The high nitrogen content (König, '03) of the mushroom, usually found to be above 5 per cent and sometimes as high as 10 per cent, led early writers to regard it as an excellent source of protein. However, Böhmer (1882) found that only

[1] Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

64 per cent of the nitrogen of Agaricus campestris was precipitable with $Cu(OH)_2$. He therefore considered the remaining 36 per cent to be non-protein in character. Mörner (1886) reached a similar conclusion using solubility in 85 per cent alcohol as a criterion for non-protein-nitrogen. Of the total nitrogen in samples of Agaricus campestris (cap 7.38 per cent and stem 6.02 per cent nitrogen), he found only 66 per cent insoluble in 85 per cent alcohol. Of this protein-nitrogen, 25 per cent was not digested in vitro by successive treatments with gastric and pancreatic extracts, indicating an 18 to 20 per cent content of digestible protein, calculated on the dry basis. Mendel (1898), using Mörner's technic with mushrooms of lower nitrogen content, found the alcohol insoluble digestible protein of several varieties to be less than 10 per cent of the total dry tissue. In attempting to characterize the non-protein nitrogen he pointed out that Winterstein (1894) was unable to make a nitrogen-free 'cellulose' preparation from Boletus edulis. Winterstein's purified 'cellulose' preparation contained 5.5 per cent nitrogen, and upon hydrolysis yielded mainly glucosamine. Mendel suggested that a considerable part of the nitrogen in mushrooms probably exists in this form.

The measurement of the digestibility of mushroom nitrogen by actual feeding trials was attempted by Saltet (1885). He found that a normal human was able to digest 69 per cent of the nitrogen in a sample of Agaricus campestris. Recently Skinner, Peterson and Steenbock ('33) found that albino rats digested 71 per cent of the nitrogen present in a sample of the same variety.

The available data concerning the vitamin B content of mushrooms are confusing, since they fail to differentiate clearly the various B components. Orton, McCollum and Simmonds ('22) fed a sample of dried Agaricus campestris at a 9 per cent level to two rats which had ceased to grow on a diet lacking in the vitamin B complex. The growth response was immediate, with an average weekly gain of 10.8 gm. per rat over a 5-week period. Hara ('23) found Boletus edulis

fully as effective as a high grade of yeast in restoring growth in rats on a ration deficient in 'vitamin B.' Weekly growth rates of 13 to 16 gm. were obtained with daily supplements of 0.3 to 0.5 gm. of dried mushrooms over a 3-week period. A sample of Agaricus campestris was only slightly inferior. Scheunert and Reschke ('31) in their recent survey of the vitamins in mushrooms have reported assays of several varieties, both in raw and cooked condition. They concluded that mushrooms are deficient in vitamin B complex, particularly in the vitamin B_2(G) component. Three to 6 gm. of either raw or cooked material were found necessary to produce an appreciable growth response in depleted rats. Unfortunately they failed to state the ingredients of their basal diets.

The foregoing review clearly illustrates the desirability of further investigating the nutritionally important components of the mushroom. It was with the hope of clarifying our knowledge of the nutritional properties of mushroom protein, in particular, that the present research was undertaken. Complications in the direct attack, which made their appearance early in the work, led to a detailed investigation of the vitamin B complex.

EXPERIMENTAL

General technic. The common mushroom, Agaricus campestris, has been used throughout these experiments. Commercial 'buttons' about 1 to 2 inches in diameter were purchased, usually in 100-pound lots. In early experiments the mushrooms were chopped, frozen immediately, and stored in this condition until needed. Each week a portion of the frozen material was finely ground, placed in a thin layer before a fan to remove a part of the water, and then incorporated into the diets. Under this treatment the tissue turned dark brown in color. It was soon found that a much better appearing product resulted if the fresh mushrooms were sliced and spread in thin layers before a fan to dry at room temperature. The dried material could then be finely ground and stored at

room temperature without difficulty. However, the samples prepared in these two ways revealed no apparent difference in nutritive properties. The casein used was a commercial acid casein which had been purified by repeated washing for a week with water slightly acidified with acetic acid.

Some 200 male rats have been utilized in these experiments. Except when otherwise stated they were placed on experiment at weaning age (3 to 4 weeks) at a weight of from 50 to 60 gm. They were housed in individual cages with raised ½ inch mesh screen bottoms. Daily food consumption records were maintained for all animals.

In reporting the data we have adopted a uniform method as follows. All weights of mushrooms as well as other materials are given calculated to a dry basis. All supplements to basal diets have been made by replacing an equal weight of starch. Diets bear the same numbers as the groups to which they were originally fed. The units in all figures (group 23 is the only exception) are so chosen that the ratio of abscissa to ordinate is constant, regardless of scale. The numbers in parentheses accompanying each curve give the number of animals used to arrive at the average values of the graph.

Protein studies. Four groups of rats were fed diets containing 50 per cent of mushrooms, with and without added protein. Samples of the mushrooms themselves averaged 6.18 per cent nitrogen. The composition of the diets is given in table 1. All animals in groups 1 and 2 were fed ad libitum. The diets of groups 3 and 4 were constructed so as to contain respectively 5 per cent of wheat and corn proteins, in addition to the protein contained in the mushrooms. The animals on these two diets were kept on an equalized food intake determined by those rats, within their groups, which had the lowest intake.

The results of these experiments are presented in figure 1. It will be seen that when mushrooms served as the sole source of protein, as well as the sole source of vitamin B, the animals failed to grow. All died within 37 days. The addition

of 5 per cent of wheat or corn protein, or even 18 per cent casein, gave only slightly better results. The apparent improvement resulting with diet 2 as rats increased in age led us to start six more animals weighing in excess of 70 gm.,

TABLE 1

Composition of diets

CONSTITUENTS	DIET NUMBER							
	1	2	3	4	5	6	7	12
Mushrooms (dry basis)	50	50	50	50				10
Cornstarch (autoclaved)	44	26			76	68	48	66
Salts 40	4	4	4	4	4	4	4	4
Cod liver oil	2	2	2	2	2	2	2	2
Casein (purified)		18			18	18	18	18
Yellow corn			40					
Corn gluten			4					
Wheat				44				
Yeast (autoclaved 5 hours)						8		
Butterfat							8	
Wheat extract dried on starch							20	

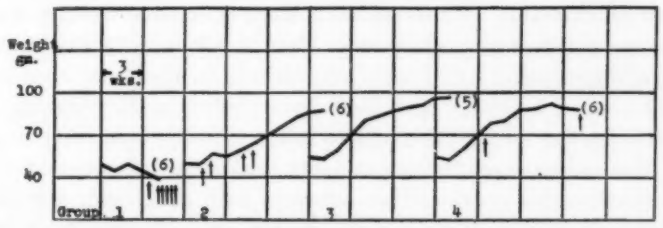

Fig. 1 Fifty per cent mushrooms as a source of protein for growth. Groups 2, 3 and 4 received in addition casein, corn, and wheat proteins, respectively. Parenthetical numbers give the number of individuals represented by each curve. Daggers indicate deaths.

using the same diet. The results were no better than those obtained with the 50-gm. rats.

The failure to obtain satisfactory growth even with a diet containing adequate protein exclusive of that contained in the mushrooms, together with the observation that food intake was low (5 to 8 gm.), led us to suspect that the mushrooms

might be deficient in vitamin B_1 or some other member of the B complex.

Studies on the vitamin B content of mushrooms. Therapeutic methods were employed in testing the mushrooms for the vitamin B complex, and B and G individually. The diet used in assaying for B has been described by Kemmerer and Steenbock ('33). The G assay was made with the technic of Bourquin and Sherman ('31). Table 1 gives the detailed composition of the basal diets; figure 2 the average growth curves of the animals.

The first assay was made for the B complex. When growth on the basal diet had definitely failed, the animals in group 5 were given mushroom supplements to the extent of 10 per cent and 25 per cent, respectively, of their diet. The graphs show that only a temporary growth response at a slow rate was obtained on either level. This failure led us to assay for B and G.

It will be seen from figure 2 that the B-depleted animals (group 6) responded somewhat to both 2 per cent and 5 per cent mushrooms. These animals were continued several weeks beyond the test period shown. Between 10 and 14 weeks after supplementation was begun all animals on the 2 per cent level had ceased to grow and died from polyneuritis; during the same period no polyneuritic symptoms were observed in any of the animals on the 5 per cent level. A 10 per cent level of mushrooms produced an average gain of 21 gm. per week. Since no better response was made on a 25 per cent level it was concluded that a diet containing 10 per cent mushrooms supplied an ample amount of vitamin B for growth.

Although the Bourquin-Sherman diet has the shortcoming that it does not produce consistently the skin changes which are considered by many to be due to a vitamin G deficiency, nevertheless this diet was believed to be the most satisfactory one available. Animals confined to this diet ceased to grow within 4 to 6 weeks but seldom exhibited clear cut symptoms of dermatitis such as sores about the mouth and paws. Supplementation of the diet with 2 per cent of mushrooms did not

improve growth consistently; neither did it intensify the skin symptoms. However, the addition of 5 per cent or more produced satisfactory growth; about 20 gm. per week, and quickly restored the animals to normality.

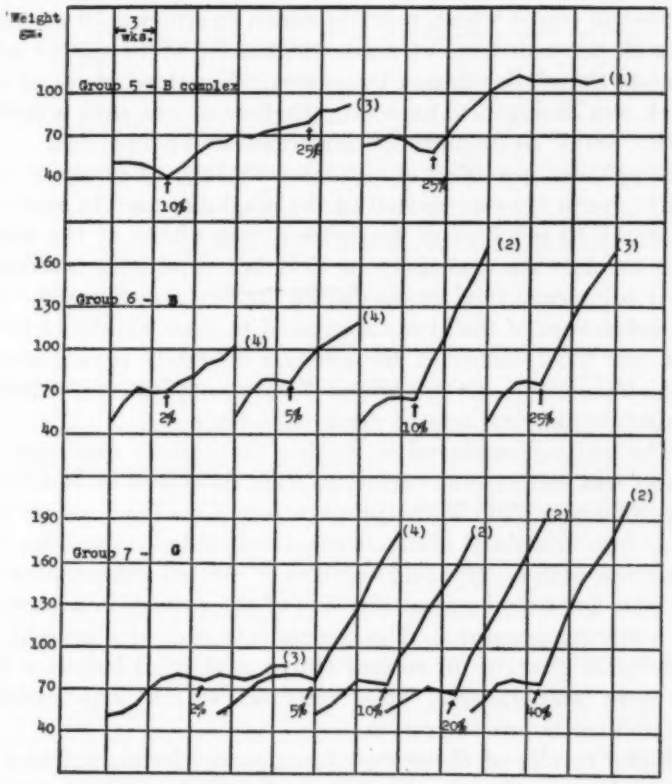

Fig. 2 Mushrooms as a source of the vitamin B complex, and of vitamin B and G. Arrows and percentages indicate mushroom supplements. Parenthetical numbers give the number of individuals represented by each curve.

It was concluded from these data that a diet containing 10 per cent of mushrooms supplies adequate amounts of both vitamins B and G. The results obtained with group 5 indicate,

however, that this level, when animals were dependent upon it as the sole source of the B complex, was by no means sufficient to produce normal growth. It appeared, therefore, that some factor, other than B or G, which was present in autoclaved yeast as well as in an alcoholic (80 per cent by weight) extract of whole wheat, must be added to either a 10 per cent or a 25 per cent level of mushrooms in order to supply adequately the whole vitamin B complex. The third phase of the work was designed to ascertain whether or not such a factor exists, and if so to study its occurrence and properties.

Supplementary effect of various materials. A group of animals (group 12) was started on the basal diet used in group 5, to which 10 per cent of mushrooms was added at the start. The animals ate sparingly at first but gradually increased their subnormal food intake during the first 6 or 8 weeks. Although a part of the group continued to grow slowly (4 to 10 gm. per week) many of the animals definitely ceased growing. These were given various dietary supplements of crude materials and extracts of crude materials.

The method employed in making the various alcoholic extracts was, with minor variations, that described by Bourquin and Sherman ('31) in the preparation of a wheat extract for their low vitamin G diet. It consisted of two successive extractions with several-fold volumes of alcohol (80 per cent by weight) followed by concentration of the extract in a vacuum at a low temperature. The concentrate was then poured on a suitable quantity of cooked starch and dried before a fan at room temperature. This 'activated starch' was incorporated directly into the ration.

Some results of these experiments are shown in figure 3. It will be seen that the dried liver and alfalfa extract proved to be very potent supplements. The wheat extract, autoclaved yeast, and brain tissue were slightly less effective. Although rat 79 failed to respond to the liver residue, a definite response was obtained when 2 per cent of the adsorbate from rice polishings replaced the 10 per cent liver residue. This led us to try a sample of crystalline B which had been

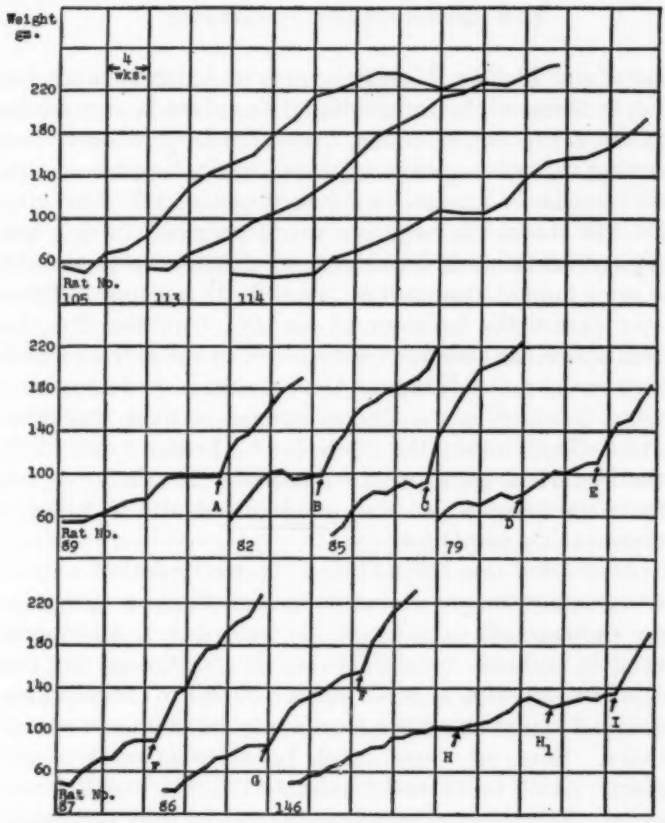

Fig. 3 The efficacy of various materials in supplementing diet 12, which contained 10 per cent of mushrooms as the sole source of vitamin B complex. The letters and arrows designate the supplements which are correspondingly listed below.

A, alcoholic (80 per cent by weight) extract of whole wheat in amount equivalent to 50 per cent of wheat in the diet.

B, 10 per cent Anheuser-Busch yeast (autoclaved 5 hours at 15 pounds' pressure).

C, 10 per cent vacuum dried fresh hog's liver.

D, 10 per cent of the autoclaved (10 hours) residue of fresh hog's liver from which the juice had been pressed.

E, 2 per cent of Fuller's earth adsorbate from rice polishing (Williams' B concentrate).

F, alcoholic extract of dried green alfalfa in amount equal to 20 per cent dry alfalfa in the diet.

G, 10 per cent dried fresh hog's brain (dried at 45°C.).

H, 2.5 gamma of crystalline B (Ohdake) daily (discontinued at H_1).

I, 5 per cent vacuum dried fresh hog's liver.

obtained from Ohdake, 2.5 micrograms of which is equivalent to at least 20 mg. of the International Standard B preparation in vitamin B potency (Ohdake, '34). It had previously been found that 2.5 micrograms of these crystals gave a good growth response in an assay conducted with diet 6. The curve for rat 146 shows the negative result obtained in this test. Subsequent addition of 5 per cent of dried liver to the diet of the same animal produced a very definite response. Space does not permit the inclusion of graphs of all the data, but the results were as follows. Autoclaved wheat extract was as effective as the raw substance. Alcoholic extract of autoclaved yeast was inactive, and a similar extract of liver was somewhat less effective than the whole liver. Lower levels of the various materials gave poorer responses. Both 5 per cent alfalfa extract and 5 per cent whole dried alfalfa failed to give appreciable responses.

The curves for rats 105, 113 and 114 are typical of animals which continued to grow slowly on diet 12 even though no dietary supplements were given. In no case was coprophagy observed in animals receiving this diet. During the long period of the experiment these animals failed to develop gross pathological symptoms other than a general sluggishness and inactivity. Some of those which failed to grow developed priapism. At 39 to 40 weeks animals 105, 113 and 114 were autopsied. The internal organs appeared entirely normal.

The failure of these and numerous other animals receiving the same diet to cease growing or to show a definite pathology was somewhat puzzling. Strong evidence against the existence of a toxic factor was shown by the apparently normal condition of animals after long periods of mushroom feeding, as well as by their continued well-being on high intakes of mushrooms following supplementation with active materials. The low food consumption offered some evidence that the mushrooms lacked palatability. However, low food intake is also known to result with diets which are deficient in known nutritive essentials. The readiness with which the B- or G-depleted animals consumed large quantities of ration containing 25 per cent or 40 per cent of mushrooms, together

with the high supplementary value of such materials as alfalfa extract or fuller's earth adsorbate as against that of pressed liver residue, are further evidences opposing the view that mushrooms made the diets unpalatable. Attempts to improve the palatability and consumption of diet 12 by the addition of 10 per cent of butterfat were without success. Substitution of sucrose for cornstarch in the basal diet led only to greater variability of weekly gains.

Fractionation of the mushrooms by extraction. Booher ('33) found that a substance which would restore growth and cure dermatitis in rats suffering from G-deficiency was soluble in 94 per cent alcohol. Since, likewise, B can be extracted readily from wheat with 80 per cent alcohol, it was surmised that an extraction of mushrooms with alcohol might remove vitamins B and G and leave behind a considerable part of the factor which we were attempting to study. Accordingly, an alcoholic extract of dried mushrooms was made in the same manner as has been described for other materials. However, subsequent assays revealed that B and G remained almost quantitatively in the extracted mushrooms. A level of the extract equivalent to 25 per cent of mushrooms in the diet was found insufficient to give a growth response with either B- or G-depleted animals.

A water extract of the mushrooms was then prepared as follows: 1 kilo of dried and finely ground mushrooms was suspended in 5 liters of water and the mixture brought to boiling. After standing for half an hour the viscous material was filtered with a Büchner funnel, and the residue suspended in 3 liters of water and filtered again. The combined filtrate was reduced to a small volume in vacuo and dried on 1.5 kilos of dried cooked starch before a fan at room temperature. The dried material contained about 40 per cent of the original weight of the dried mushrooms. It was fed as a supplement to basal diet 5 at levels equivalent to 10 per cent, 20 per cent, and 40 per cent of mushrooms. The results are presented in table 2. They will be discussed in connection with other phases of the work which are presented in the same table.

Further studies on supplementary substances; relationships between growth rate and amount of food consumed. A series of trials was made in which various levels of dried yeast and mushrooms were fed, both singly and in combination with each other. Diet 5 was used as the basal diet. The results are summarized in table 2. During the 10-week period group 8, which received 8 per cent of dried yeast, reached an average weight of 299 gm., representing a weekly gain of 24.7 gm. per rat. Both food consumption and growth rate were markedly less when 10 per cent of mushrooms was fed together with 8 per cent of yeast (group 9). Group 12A, which received 10 per cent of mushrooms alone (diet 12), performed still less satisfactorily. Although an apparent slight advantage was obtained with 20 per cent of mushrooms, 40 per cent was inferior to either level, with no improvement resulting from the addition of 8 per cent of yeast. The data presented for groups 15, 16 and 17 show, however, that a reverse situation obtained with the water-soluble fraction of mushrooms. Animals receiving this material at a 10 per cent equivalent level definitely failed. Increasing levels gave increasing growth rates, the 40 per cent level being superior to any of the other diets containing only mushrooms as a vitamin B source.

The effectiveness of various B preparations in supplementing diet 12 was further investigated. A comparison of the data for groups 12a and 18 reveals that here, again, as with rat 146 above, no advantage was shown by rats receiving 2.5 micrograms of Ohdake's crystalline vitamin B. An acid-alcohol extract of yeast (Itter, Orent and McCollum, '35) fed at a level equivalent to 4 per cent of yeast in the diet, was only slightly better. An apparent improvement was obtained with groups 19 and 20 which received the International Standard B preparation and a Burroughs-Wellcome B concentrate from rice polishings, respectively, in amounts equivalent to 2 International units of vitamin B. The slightly better results obtained with the crude B carriers appeared not to be highly significant. Unfortunately the limited supply of materials prevented us from feeding them at higher levels. At the

termination of these trials one rat in group 20 was given diet 12 alone. During the next 4 weeks, its body weight fell 11 gm. The rice-polishings concentrate was then fed in a separate container at a level which was tenfold that previously fed. During the following 3 weeks the animal doubled its food intake and gained 79 gm. (26 gm. per week).

For comparison with other data presented, the mean growth rate of fifty-seven animals which had been confined to diet 12 is included in the table.

A comparison was made between diet 12 and several satisfactory growth-promoting rations in equalized feeding trials. The food intake of animals in group 11 was controlled by that of

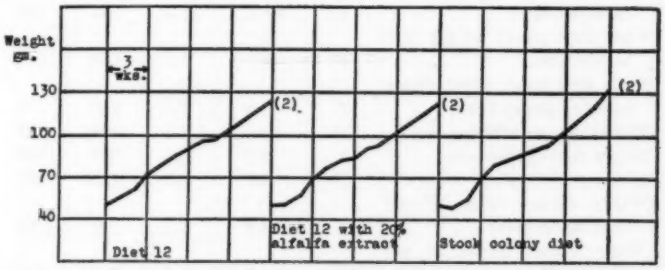

Fig. 4 A comparison of growth in equalized feeding experiments. Food consumption was controlled by the animals receiving diet 12.

group 12a; likewise in group 22 the food intake of animals receiving diet 12 determined the intake of those receiving other diets. It will be seen from the results shown in table 2 and group 22 in figure 4 that no marked differences in growth occurred, indicating that quantity rather than quality of the diet was the deciding influence upon growth. These data are only further verification of similar evidence shown in table 2, that those animals which ate the most food showed the best gains.

The curves for group 23 shown in figure 5 throw some additional light upon this subject. Animals weighing about 40 gm. each were placed in individual cages and received the stock colony diet (Steenbock, '23) for 6 days. By this time

all had become completely accustomed to their new environment and were eating 10 gm. of food daily. The diet was then changed. Two animals received diet 8 (8 per cent yeast) and

TABLE 2

Comparison of growth on mushroom, yeast and supplemented mushroom diets. Experimental period 10 weeks

GROUP NO.	SUPPLEMENT TO BASAL DIET 5	NUMBER OF RATS	FOOD CONSUMPTION (AVERAGE GRAMS PER RAT PER DAY)	MEAN BODY WEIGHT		
				Weekly gain per rat	Maximum	Final
				gm.	*gm.*	*gm.*
8	8 per cent yeast	3	13.5	24.7	299	299
9	8 per cent yeast; 10 per cent mushrooms	2	8.1	13.5	185	185
10	8 per cent yeast; 40 per cent mushrooms	3[1]	4.8	5.3	104	104
11	8 per cent yeast (food intake same as group 12a)	3	5.5	7.8	136	135
12a	10 per cent mushrooms	3	5.5	6.3	119	116
13	20 per cent mushrooms	3	5.4	8.3	136	136
14	40 per cent mushrooms	3	4.9	4.9	102	102
15	Water extract from 10 per cent mushrooms	3	4.8	3.8	99	91
16	Water extract from 20 per cent mushrooms	3	6.5	9.4	148	148
17	Water extract from 40 per cent mushrooms	3	6.7	10.9	161	161
18[2]	10 per cent mushrooms; 2 units B_1 crystals (Ohdake)	2	5.8	7.0	116	111
19	10 per cent mushrooms; 2 units Int. Std. B_1	3	6.8	11.0	160	160
20	10 per cent mushrooms; 2 units Burroughs-Wellcome B_1 concentrate	3	7.2	11.5	165	165
21	10 per cent mushrooms; 4 per cent yeast extract (Itter)	2	6.6	8.9	140	140
12[2]	10 per cent mushrooms	57		5.0		95

[1] One animal died at 5 weeks.

[2] Nine-week period.

the other two received diet 12 for the following week. Average food consumption and body weight of each pair are shown in the figure. The animals receiving yeast continued to consume over 10 gm. of food and grew normally. Those receiving

mushrooms consumed successively an average of 8, 5 and 4 gm. of food during the consecutive days following the change of diet. Had the mushroom-containing diet proven merely unpalatable the animals would probably have eaten less than 8 gm. during the first day on the diet. A more plausible explanation might include an adverse gastro-intestinal reaction to the diet, followed by a diminished appetite for mushroom-containing food.

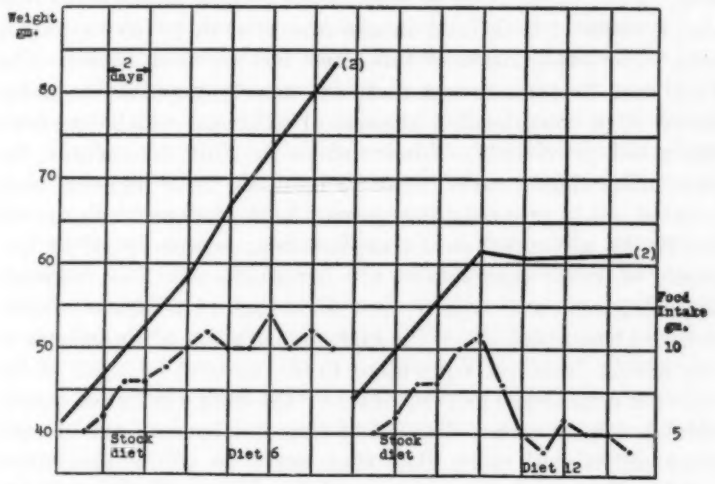

Fig. 5 Simultaneous food consumption and growth curves during transition from the stock diet to yeast and mushroom diets.

An attempt was then made to study the motor activity of the alimentary tract after consumption of mushroom and yeast diets. Six 200-gm. rats which were receiving the stock colony diet were starved for 36 hours. Each was then given 6 gm. of food, three receiving diet 8 and the three others diet 12. After 1½, 3 and 5 hours one animal on each diet was killed, the contents of stomach and upper three-quarters of the small intestine removed, dried and weighed. No significant differences were found. After 5 hours 20 to 25 per cent of the meal still remained in the stomach in both cases; the quantity found in the intestine was constant and of small magnitude.

DISCUSSION

From the preceding data it follows that a study of the nutritive properties of mushrooms with rats is beset with difficulties, of which the subnormal consumption of the ration is outstanding. This latter effect was especially marked when mushrooms were included in the diet from the beginning of an experiment. It is well exemplified by the effect of even a low percentage of mushrooms in an otherwise satisfactory diet. The introduction of only 10 per cent of mushrooms into diet 8 reduced both food intake and growth by 40 to 45 per cent. The explanation of this effect has not been found. The likelihood that mushroom diets are merely unpalatable is opposed by a considerable amount of evidence which has been discussed previously. Observations tending to exclude the possibility that a toxic factor is involved have likewise been pointed out in previous discussion. Lack of digestibility could hardly be advanced as an explanation, especially when low levels of mushroom intake are involved. Further evidence opposing such a view is the low fiber content (Skinner, Peterson and Steenbock, '33), the high digestibility of the nitrogen-containing fraction (equivalent to 30 per cent or more of the whole if considered as protein), and the high content of water-soluble constituents. The belief that mushrooms are of such poor nutritional value that they serve to dilute the ration can hardly be upheld; evidence shown in equalized intake experiments with different diets is quite to the contrary. Failure to find gastro-intestinal irritation or gaseous accumulation in autopsied animals would tend to eliminate the possibility of such disturbances as causes of diminished food consumption.

In the studies of the vitamin B content of mushrooms considerable evidence has indicated that some factor of the vitamin B complex, other than B or G is lacking, or present in only small quantity. This factor would seem to be heat stable and closely associated with vitamin B. However, this evidence cannot be accepted as final since the reactions may be clouded by the effects of the poor consumption of mushroom diets.

A study of the mushroom protein becomes involved in the same difficulties. The complete failure of growth and the short survival period of animals receiving mushrooms as their only protein source, when compared with the effect of the addition of casein to the same diet, would suggest a deficiency in the mushroom protein. However, the experiments with the water-soluble fraction of the mushrooms lend no encouragement to a further study of the protein fraction. Better consumption and growth-promoting properties were shown with a 40 per cent level of the water-soluble fraction than with an equivalent amount of the whole mushroom. Most of the protein, as well as the factor which is responsible for poor food consumption, would likely appear in the insoluble material. Although no studies have been made with this insoluble fraction it is probable that it, like the whole mushrooms, would not be consumed in satisfactory quantities.

It is apparent from our studies with rats that the mushroom does not serve as a particularly valuable dietary constituent. It is a fair source of vitamin B and ranks favorably with milk and yeast as a carrier of vitamin G. Limited data tend to show that the protein is incomplete. While it is shown that subnormal food consumption and growth follow the introduction of as little as 10 per cent mushrooms into an otherwise satisfactory diet, there is no evidence that this effect is due to toxicity.

It is unlikely that these findings are of significance in practical human nutrition. In the great majority of cases mushrooms constitute only a small part of an occasional meal and are included in the human dietary largely because of their 'condimental value' rather than for their nutritive properties.

SUMMARY

The nutritive properties of the mushroom Agaricus campestris have been studied with albino rats.

Diets which contained mushrooms were consumed in subnormal quantities, and consequently growth was subnormal on these diets. Exceptions to this general result were ob-

served when mushrooms were fed to animals which were depleted in vitamin B or G.

Mushrooms were found to be a relatively good source of vitamins B and G. Levels of 10 per cent and 5 per cent of the diet on a dry weight basis supplied sufficient B and G, respectively, to support satisfactory growth.

The data indicate that a diet containing 10 per cent of mushrooms as the only source of vitamin B is deficient in some factor other than B or G.

A preliminary study has indicated that mushroom protein is incomplete.

A detailed investigation of these deficiencies is hampered by the poor consumption of diets which contain mushrooms.

There is no evidence to show that Agaricus campestris contains a toxic principle.

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THE EFFECT OF THE INGESTION OF SALINE WATERS UPON THE pH OF THE INTESTINAL TRACT, THE NITROGEN-BALANCE AND THE COEFFICIENT OF DIGESTIBILITY

V. G. HELLER, J. R. OWEN AND LUCILE PORTWOOD

Department of Agricultural Chemistry Research, Oklahoma Experiment Station, Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma

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In previous articles from this laboratory (Heller, '32 and '33; Heller and Paul, '34) attention has been called to the occurrence of saline and alkaline waters over large areas of the earth's surface, and to the injurious effect of these waters upon domestic animals when used in excessive concentrations for drinking purposes. While rather specific concentrations have been established as the maximum amount permitting growth, reproduction, and general well-being of animals no definite cause has yet been determined as being responsible for the deaths and injuries noted although possible reasons were offered in the articles cited.

It has frequently been suggested that the ingestion of large quantities of salts and alkalies would seriously alter the pH of the stomach and intestinal tract through their buffering action and possibly poor assimilation might result in the inability of an animal to gain weight under these conditions. A series of tests have been carried out in which the coefficients of digestibilities and nitrogen-balances were determined to ascertain if osmotic pressure, assimilation, or secretion might be altered and the poor response to food intake be due to poor absorption of the nutrients. In a similar series of tests the pH of the entire intestinal tract has been determined at stated intervals comparing rats receiving distilled water with others

receiving varying concentrations of different salt solutions. This work has been in progress for several years and the results obtained are briefly summarized in this article.

EXPERIMENTAL

Rats, chickens and hogs have been used in these tests. Rats were used far more extensively, and all data recorded will be for this species excepting that for hogs shown in table 3. The animals used were of vigorous stock from our colony. The basic ration which had been used for years, consisted of mixed cereals amply supplemented with animal proteins, minerals and vitamins. The metabolism cages used were of our own design which have frequently been improved during the past 15 years. Though no cages used for the rats represent perfect recovery, this model approaches an ideal.

In all of the tests, groups of comparable rats were divided into similar groups of four or more and placed in round metal cages. The basic ration was fed ad libitum. The drinking solutions were prepared by dissolving given amounts of pure salts in distilled water in the amounts indicated and given to the animals in inverted vacuum fountains. In every case the growth of the individual rats, the amount of solution drunk daily, as well as the weight of the food consumed were recorded. The animals were kept under constant observation from 4 to 10 weeks previous to the digestion tests. They were than placed in the metabolism cages at least 3 days prior to the time samples were taken to permit the animals to adjust themselves to their new environment. During the time the tests were in progress, the exact amount of food eaten and the drinking solution consumed were recorded daily. The feces were collected several times daily and quickly dried. The urine was preserved with H_2SO_4 and made up to definite volume. The feed, feces and urine were analyzed by the usual accepted methods (condensed statements of which may be found in Peters and Van Slyke, '31) and the nitrogen-balances and digestibilities calculated and recorded. The trials have been repeated with different sets of rats and for

different years. Figures presented represent the average of 24 rat days in most cases.

Table 1 represents the averages of several hundred trials with young mature rats. The feed for each group being similar, but the drinking waters varied from distilled water in one case, to various salt solutions in different concentrations as indicated. These tests were conducted during the winter months.

TABLE 1

The nitrogen-balance and the apparent coefficient of digestibility of rats receiving saline drinking waters

DRINKING WATER	NITROGEN BALANCE (MILLIGRAMS PER PERIOD)	APPARENT COEFFICIENT OF DIGESTIBILITY				
		Protein	Ash	Fat	Fiber	Nitrogen free extract
Distilled water	+ 47.3	62.3	28.3	81.6	15.2	82.3
NaCl 1 per cent	+ 34.5	71.5	53.2	87.3	17.8	80.6
NaCl 1.5 per cent	+ 32.5	75.2	68.9	85.6	28.1	81.1
NaCl 2 per cent	+ 30.5	75.2	76.5	81.8	27.1	81.5
$MgSO_4$ 1 per cent	+ 34.5	71.4	42.9	85.6	20.6	79.7
$MgSO_4$ 1.5 per cent	+ 40.6	73.3	59.1	87.2	26.6	79.5
$MgSO_4$ 2 per cent	+ 30.4	65.5	58.0	79.4	17.7	80.1
$CaCl_2$ 1 per cent	+ 42.5	71.8	41.4	84.1	25.3	80.1
$CaCl_2$ 1.5 per cent	+ 40.0	72.6	47.6	85.9	21.9	77.9
$CaCl_2$ 2 per cent	+ 41.1	66.7	51.6	76.3	28.5	80.1

Table 2 represents a similar series of results using younger animals during the spring months. The only similarity between the two groups being the manner of conducting the experiment.

TABLE 2

Apparent coefficient of digestibility and nitrogen-balance of rats consuming basic ration and drinking saline water

WATER CONSUMED	NITROGEN BALANCE (MILLIGRAMS PER PERIOD)	APPARENT COEFFICIENT OF DIGESTIBILITY				
		Protein	Ash	Fat	Fiber	Nitrogen free extract
Distilled water	+ 17.1	76.8	38.6	77.9	16.7	88.5
Saturated $Ca(OH)_2$	+ 16.6	77.6	28.4	79.2	16.6	85.7
Saturated $CaSO_4$	+ 25.3	76.6	32.9	78.2	18.2	86.8
1.5 per cent $CaCl_2$	+ 22.2	76.3	24.0	82.1	20.7	87.7
1.5 per cent $MgSO_4$	+ 24.3	78.9	29.9	84.1	21.4	86.0

Table 3 records similar data, in this case for hogs.

TABLE 3

Apparent coefficient of digestibility and nitrogen-balance of hogs drinking distilled and salty waters

WATER CONSUMED	NITROGEN BALANCE (MILLIGRAMS PER PERIOD)	APPARENT COEFFICIENT OF DIGESTIBILITY				
		Protein	Ash	Fat	Fiber	Nitrogen free extract
Normal	+ 42.7	71.7	28.7	59.5	15.8	74.3
1 per cent NaCl	+ 48.5	81.5	56.8	73.4	42.3	86.1

Table 4 records similar data for animals receiving distilled water and having salts added to the basic ration. In this case distilled water was given ad libitum to all groups. The water consumption of the salt-eaters was much increased, evidently in an attempt to flush the salts from the body.

TABLE 4

Apparent coefficient of digestibility and nitrogen-balance of rats receiving varying amounts of salts added to basic ration

SALT ADDED TO BASIC RATION	NITROGEN BALANCE (MILLIGRAMS PER PERIOD)	APPARENT COEFFICIENT OF DIGESTIBILITY				
		Protein	Ash	Fat	Fiber	Nitrogen free extract
None	+ 29.7	73.1	50.6	76.5	19.4	81.8
5 per cent NaCl	+ 20.9	80.2	65.7	85.1	18.7	81.0
3 per cent $CaCl_2$	+ 18.4	72.6	48.6	84.2	15.1	80.8
5 per cent Na_2HPO_4	+ 26.5	75.2	60.8	85.5	12.6	83.2

Possibly the figures for ash should have been omitted from the standpoint of true digestibility, but they have been included as showing the trends produced by various ions.

An inspection of the data presents one fact which is contrary to what one would anticipate and that is that the maximum amount of salts that can be consumed without producing serious results is evidently not responsible for any interference of food utilization; in fact, there is considerable evidence that the presence of these salts in quantities less than the amount which produces serious disturbances, such as rapid loss of weight, inability to produce and nourish young and

even death, facilitates digestion and absorption. The concentrations of soluble salts producing the injuries referred to have been reported elsewhere as approximately 15,000 parts per million.

pH OF INTESTINAL TRACT

After the digestion trials with the animals used in the above tests were completed, the animals were then sacrificed to make a study of the effect of these salts upon the hydrogen ion concentration of the digestive tract. These animals were guillotined and samples removed from designated portions of the entire length of the intestinal tract.

The method of determining the hydrogen ion concentration of a material like feces is always debatable. Many workers have used colorimetric methods but with the large amount of buffer material present it is doubtful if it is sufficiently accurate. The electrometric, the quinhydrone and the glass electrode have been recommended and conducted by various workers. When this work was started the procedure of Redman, Willimott and Wokes ('27) seemed to offer greater advantages. At about this time, Eastman and Miller ('32, '35) reported the results of the effect of diet on the pH of the rats' intestinal contents using the glass electrode. The method used in this laboratory was one devised for our own work. The method of sampling, the amount of dilution, and the time of shaking being chosen as most favorable for these determinations. The method is briefly outlined as follows:

The intestine was opened and a sample of the contents removed with a small glass scoop, deposited in a dry test tube and 5 cc. of conductivity water added. The mixture was then macerated and 10 drops of an acetone solution of quinhydrone containing 0.036 gm. per cubic centimeter were added. A portion of the mixture was then transferred to a Bailey electrode and shaken for 3 minutes; attached to the potentiometer, and read exactly 3 minutes later. The variations in the readings produced by the amounts of material were of much less importance than the matter of time and temperature. The re-

sults of several hundred such determinations have been collected, averaged and condensed in the following table.

TABLE 5

pH of the contents of the intestinal tract of rats receiving salts and acids added to food

RATION	STOMACH	DUODENUM	JEJUNUM	ILEUM	CECUM	DESCENDING COLON
Basic A	4.82	6.55	7.27	6.94	6.95	7.26
Basic A + 3 per cent $CaCO_3$	5.38	6.26	7.86	7.39	7.37	7.49
Basic A + 5 per cent $CaCO_3$	4.66	6.07	7.19	7.10	7.19	7.29
Basic A + 3 per cent $CaCl_2$	4.01	6.55	7.92	7.28	7.36	7.52
Basic A + 4 per cent $CaCl_2$	3.11	6.37	7.39	7.05	7.20	7.25
Basic A + 5 per cent NaH_2PO_4	4.60	6.08	6.61	6.69	6.78	6.71
Basic A + 3 per cent citric acid	4.09	6.32	7.75	7.32	7.24	7.47
Basic A + 5 per cent citric acid	3.10	5.89	7.07	7.22	7.09	7.13

An inspection of the data for these rats, which were from 5 to 6 months of age, does not indicate that the intestinal content is appreciably altered by the presence of various acid and alkaline salts in the drinking supply. Calcium chloride tends to increase the acidity of the stomach but increases the alkalinity of the lower intestine.

The fact that the pH is only slightly altered by salts confirms the conclusion drawn by Eastman and Miller ('35) in regard to the effect of various diets, namely, that in most cases the food causes little changes in the pH. Possibly this statement would not hold true for animals receiving a more concentrated drinking solution. The problem is involved in that at higher concentrations the animals die and the data cannot be obtained.

CONCLUSIONS

The use of drinking waters containing considerable quantities of dissolved salts does not interfere with the nitrogen utilization of normal rations. Likewise the apparent coefficients of digestibility of the constituents of the ration are not interfered with; in fact, the trend of all determinations indicate that assimilation or digestibility is aided by the presence of reasonable amounts of salt so long as the total content is kept below the concentration where serious disturbances in growth and reproduction takes place.

The pH of the entire intestinal tract is not appreciably altered by the presence of alkali or acid salts in the drinking water, providing the concentration present does not exceed the amount permitting a somewhat normal life. Observed changes are more apparent in the stomach than in the lower intestine.

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THE DIFFERENTIAL ANTIRACHITIC ACTIVITY OF VITAMIN D MILKS [1]

ROBERT W. HAMAN AND HARRY STEENBOCK
Department of Agricultural Chemistry, University of Wisconsin, Madison

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The recent commercial acceptance of vitamin D milks, whether produced by feeding cows irradiated yeast,[2] by direct irradiation,[2] or by the addition of vitamin D concentrates, has resulted in the appearance of a considerable literature discussing their comparative antirachitic effectiveness in relation to each other and to irradiated ergosterol and cod liver oil. The stimulus for this discussion growing out of the report of Massengale and Nussmeier ('30) working with chicks, was provided by the clinical reports of Hess, Lewis and Rivkin ('30), working with infants and confirmed by De Sanctis and Craig ('30) and Barnes and Brady ('30), that the vitamin D from irradiated ergosterol was not as effective as that from cod liver oil when compared on a rat unit basis.

The majority of clinicians who have investigated the antirachitic effectiveness of vitamin D milks are of the opinion, 1) that they are very effective both for the prevention and cure of rickets when used in proper amounts, 2) that they are more efficient unit for unit than other antirachitics, and 3) that the various milks compared with each other are equally effective. Hess, Lewis, MacLeod and Thomas ('31) fed milk

[1] Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

A preliminary report of the data in this paper was presented before the American Institute of Nutrition, April 10, 1935. Similar data were presented by Bethke, Krauss, Record and Wilder, Proc. Am. Inst. Nutrition, J. Nutrition, vol. 9, suppl. p. 7, 1935.

[2] Referred to hereafter as 'yeast milk,' or 'irradiated milk.'

produced by feeding cows irradiated ergosterol and irradiated yeast to 102 infants. Both rachitic and non-rachitic infants were included in their experiments. The authors report that the lower level, 60 units[3] daily did not successfully prevent or cure rickets. However, the higher level, 120 units daily, was successful in both their preventive and curative studies. Wyman and Butler ('32) reported that yeast milk containing 160 units per quart at daily levels of 160 and 200 units initiated calcium deposition in the bones of rachitic infants within 2 weeks of the beginning of treatment. Four infants were used in this study. Hess and Lewis ('32) concluded from a study including ninety-eight infants that irradiated milk is highly effective both for the prevention and cure of rickets. The daily intake of vitamin D was 37 and 50 units. Mitchell, Eiman, Whipple and Stokes ('32) fed irradiated milk and milk from irradiated cows to thirty-four infants. The irradiated milk contained 65 units per quart and the milk from irradiated cows 22 units per quart. Both milks were reported as being successful antirachitics. Gerstenberger and Horesh ('32) fed two rachitic infants milk from cows that were fed irradiated ergosterol. The daily intake of vitamin D for the infants was 80 units. The authors concluded that satisfactory progress in healing occurred in 10 to 11 weeks. The healing was not complete at this time. Hess and Lewis ('33) in a curative study fed irradiated milk at daily levels of 28 and 42 units, and yeast milk at 60 and 90 units. They concluded that 35 to 42 units daily of irradiated milk and 70 units or more of yeast milk were sufficient for protection against rickets. The efficacy ratio of the two milks was reported as approximately 1:2. Kramer and Gittleman ('33) fed in a curative type of experiment both irradiated milk and yeast milk at daily levels for each milk of 40 and 50 units. Ten rachitic infants were included in this study. The authors could not demonstrate any significant difference between the two milks. They concluded, because of the definite curative effect of a daily intake

[3] The vitamin D potencies are expressed as Steenbock units. For all practical purposes we have found that 1 Steenbock unit is equivalent to 3½ International units.

of 40 units that vitamin D milk is a very effective antirachitic agent. Drake, Tisdall and Brown ('34) studied the antirachitic effectiveness of cod liver oil, Viosterol, and irradiated milk in a preventive study with 141 infants. No attempt was made to compare the effectiveness of these antirachitics. They reported 35 to 70 units of irradiated milk daily were sufficient for the prevention of the development of either moderate or marked rickets. Jeans and Stearns ('34) concluded from a study with seven infants that irradiated milk, cod liver oil concentrate milk, and cod liver oil and milk separately, are all equivalent upon a rat unit basis. Furthermore, that 50 units per day were insufficient for the best development of the infant although rickets may be prevented. They used as criteria, roentgenograms, calcium retention, serum Ca and P determinations, and rates of growth. Fifty units daily was the maximum vitamin D intake and at no time during the experiment did an infant develop rickets. Wyman, Eley, Bunker and Harris ('35) in a curative study fed vitamin D from irradiated milk and yeast milk at daily levels of 30 to 50, and 50 to 65 units, respectively. They used roentgenograms and the rate of increase of the Ca $\times$ P product as criteria. They concluded that the two milks unit for unit were equivalent and that vitamin D milk containing 50 units per quart will produce demonstrable healing in infants in 4 weeks.

Gerstenberger et al. ('35) in a carefully controlled curative study fed irradiated milk and yeast milk to thirteen rachitic infants. They used roentgenograms and serum Ca and P determinations as criteria. They reported that 40 units of vitamin D milk produced satisfactory changes in the bones in 10 to 12 weeks and in the blood in 49 to 62 days. In regard to the comparative effect of the two milks, they concluded that there were no practical differences although if a slight difference existed it was in favor of irradiated milk. Barnes, in two publications ('33), ('34), has reported the results of two series of experiments wherein cod liver oil concentrate milk was fed both to rachitic and non-rachitic infants. In his

first paper ('33) he reported the results of his studies with fifteen rachitic infants. They were fed vitamin D concentrate milk at daily levels of 100 and 150 units. He reported that all of the infants responded promptly. In his second paper ('34) he reported that thirty-two normal infants were protected against rickets when fed 50 units daily of cod liver oil concentrate milk. Six infants that were slightly rachitic at the beginning of the experiment showed satisfactory healing. A control group of twenty-five infants showed an incidence of rickets of 56 per cent. Roentgenograms and gain in weight were used as criteria. It was concluded that the vitamin D of irradiated milk and of cod liver oil concentrate added to milk, are equally efficient. Wilson ('34) fed cod liver oil concentrate milk in a preventive study to thirty-three infants. The daily intake of vitamin D was 90 to 140 units. The criteria consisted of monthly roentgenograms and body weights and lengths. Of the thirty-three infants, fourteen remained normal, seventeen developed slight rickets, and two a moderate degree of rickets. The authors state in the conclusion of their report that the number of units of vitamin D concentrate per quart that are necessary can be determined only by further study. Rapoport, Stokes and Whipple ('35) in both a curative and preventive study with twenty-three male Negro infants reported that irradiated evaporated milk containing 46 units per 14.5-ounce can was adequate for the prevention of rickets. They also reported that this milk was inadequate for the cure of rickets in Negro infants. Roentgenograms, gain in weight, and increase in body length were used as criteria.

The reason for the apparent greater efficiency of vitamin D milks over other sources of vitamin D has not been determined. Ansbacher and Supplee ('34) have postulated that the vitamin may be combined with a protein fraction, thus enhancing its absorption and utilization. Very recently Lewis ('35) has reported that propylene glycol as a solvent for vitamin D greatly increased its effectiveness as compared with corn oil as a solvent when added to milk and included in the

dietary of rachitic infants. Waddell ('34) believes that the high efficiency of irradiated milk can be explained on the basis that it contains the activated provitamin of crude cholesterol, which he found to be so effective with chicks and which has been shown recently by Tisdall, Drake and Brown ('35) to be as effective as cod liver oil for infants. The reported high efficiency of yeast milk, however, cannot be explained upon this basis.

Krauss, Bethke and Monroe ('32) working with chicks found that "on a rat unit basis it required four times as many vitamin D units in yeast milk butter fat as in cod liver oil to bring about the same degree of calcification." Supplee, Bender and Dorcas ('32) working with chicks found irradiated milk to be an effective agent for the prevention of rickets. However, these investigators made no attempt to compare the effectiveness of irradiated milk with any other antirachitic agent.

In our studies we have availed ourselves of the striking parallelism that exists between the infant and the chick in the directional response to vitamin D therapeusis to make a quantitative comparison of the efficiency of an equal number of units of vitamin D from irradiated milk, yeast milk, irradiated ergosterol, and irradiated cholesterol.

Briefly our data show that yeast milk is far less effective than irradiated milk for the chick; that irradiated milk, irradiated cholesterol, and cod liver oil are of approximately the same order of effectiveness; and that the constituents of milk as a vehicle for vitamin D do not increase its effectiveness.

EXPERIMENTAL AND RESULTS

Day-old White Leghorn chicks, obtained from commercial hatcheries, were placed in groups of fifteen in brooders with wire screen bottoms. They were given a rachitogenic diet (Hart, Kline and Keenan, '31) with additions of various amounts of vitamin D. At the end of 5 weeks they were killed, both tibia were removed and analyzed for ash after extraction with alcohol.

In this type of experiment to obtain results that are significant, it is necessary to feed levels of vitamin D that will produce a rickets of moderate or slight severity. To insure securing the appropriate levels of intake of vitamin D, preliminary assays using the line test were carried out with five rats at each level of intake and in most instances prophylactic assays using at least nine animals at each level of intake were carried out as well.

In our first series we determined the comparative antirachitic effectiveness of 'irradiated milk,' 'yeast milk,' irradiated ergosterol, and cod liver oil. For these determinations the cod liver oil and irradiated ergosterol were diluted with Wesson oil so that the desired level of intake of vitamin D was obtained when the oil was added to the extent of 1 per cent of the diet. The 'irradiated milk' and 'yeast milk' were diluted with untreated milk so that the effective intake of vitamin D was obtained with the consumption of 90 cc. of milk daily. This amount was thoroughly mixed into 97 gm. of the basal ration. For control purposes untreated milk was included in the rations of the cod liver oil and irradiated ergosterol groups in the same proportion. The rations were fed to the chicks twice daily.

The results of this series presented in table 1 reveal the relative inefficiency of yeast milk as compared with both cod liver oil and irradiated milk. Ten units of vitamin D from yeast milk produced no evidence of protection, whereas 2.5 units given either as cod liver oil or as irradiated milk revealed a demonstrable effect.

The data in table 1 also show that apparently irradiated milk was not so effective as cod liver oil. However, the differences are small and well within the range of experimental error as the unitage administered had been determined only by means of the line test on five animals.

That irradiated milk has a high efficiency per unit of vitamin D in clinical tests has already been discussed. On the supposition that the high efficiency might be due, at least in part, to the favorable effect of the constituents of milk other

than the vitamin prompted us to feed milk to all of our groups in this experiment. The results suggest that irradiated milk has no greater efficiency per unit than cod liver oil.

A second series of experiments was carried out in an attempt to confirm our first results and to determine the factors responsible for the greater effectiveness of a unit of vitamin D in irradiated milk than in 'yeast milk.' We investigated the possibility that this difference could be assigned to either the skimmed milk or the butter fat.

TABLE 1

Series I. Ash in the tibia of chicks fed vitamin D from cod liver oil, irradiated ergosterol, irradiated milk and yeast milk

SUPPLEMENTS TO THE BASAL RATION	STEENBOCK UNITS PER 100 GM. RATION	NUMBER OF CHICKS	AVERAGE WEIGHT OF CHICKS	AVERAGE WEIGHT OF ASH	AVERAGE ASH
			gm.	*gm.*	*per cent*
Untreated milk	0	13	166	0.2009	31.73
Untreated milk + cod liver oil	2.5	13	191	0.2217	36.08
Irradiated milk	2.5	15	193	0.2215	33.69
Yeast milk	2.5	12	145	0.1692	32.33
Untreated milk + cod liver oil	5.0	13	212	0.2570	38.35
Irradiated milk	5.0	10	232	0.2981	36.26
Yeast milk	5.0	13	161	0.2025	32.72
Yeast milk	10.0	14	156	0.1693	32.60
Untreated milk + irradiated ergosterol	12.5	13	182	0.1961	32.93
Untreated milk + irradiated ergosterol	50.0	13	223	0.2672	37.55
Untreated milk + irradiated ergosterol	100.0	13	217	0.3433	44.57

For this purpose cream was separated, respectively, from yeast milk, irradiated milk and untreated milk. The cream was churned to butter and the butter fat was melted, decanted, and filtered. The skimmed milk was preserved by freezing. Both the curative line test method and the prophylactic method with analysis for bone ash were used to assay the butter fats. The line test was used for preliminary results and the prophylactic test with ash analyses for the final values.

In the chick rations the amount of butter fat and skimmed milk was kept constant. Every 2 kg. of ration contained 80

gm. of butter fat. Immediately before feeding 100 cc. of skimmed milk were added to each 100 gm. of ration. To attain this constancy, dilutions with untreated skimmed milk and untreated milk butter fat were obviously necessary. To determine the influence of the skimmed milk fraction the experimental groups were arranged so that the skimmed irradiated milk was fed with butter fat from yeast milk and

TABLE 2

Series II. Ash in the tibia of chicks fed vitamin D from cod liver oil, irradiated milk butter fat and yeast milk butter fat, together with a constant amount of skimmed milk

SUPPLEMENTS TO THE BASAL RATION	STEENBOCK UNITS PER 100 GM. RATION[1]	NUMBER OF CHICKS	AVERAGE WEIGHT OF CHICKS	AVERAGE WEIGHT OF ASH	AVERAGE ASH
			gm.	*gm.*	*per cent*
Untreated milk butter fat + untreated skimmed milk	0	13	204	0.2152	33.92
Cod liver oil + untreated milk butter fat + untreated skimmed milk	2.5	13	231	0.2532	37.72
Irradiated milk butter fat + untreated skimmed milk	2.0	14	247	0.2671	35.22
Yeast milk butter fat + untreated skimmed milk	3.6	14	196	0.2020	32.61
Cod liver oil + untreated milk butter fat + untreated skimmed milk	5.0	12	251	0.2884	42.11
Irradiated milk butter fat + untreated skimmed milk	4.0	12	258	0.3231	39.62
Yeast milk butter fat + untreated skimmed milk	7.25	11	201	0.2418	34.28
Irradiated milk butter fat + yeast skimmed milk	4.0	14	265	0.3340	40.60
Yeast milk butter fat + irradiated skimmed milk	7.25	11	194	0.1948	32.28

[1] Unitage determined by prophylactic assays with rats.

vice versa. In other groups untreated skimmed milk was fed with both butter fats. Skimmed milk and butter fat from untreated milk were added to the rations of both the negative and positive control groups.

The data presented in table 2 reveal that the yeast milk butter fat afforded no protection at the levels fed. The addition of skimmed irradiated milk or skimmed control milk

did not increase its effectiveness. The irradiated milk butter fat, regardless of the skimmed milk supplement afforded approximately the same degree of protection as cod liver oil.

The results of this series therefore confirmed our previous findings in regard to the difference in action of the vitamin D from the two milks, and furthermore, assigned this difference to the butter fat fraction of the milk.

We felt, however, that it was desirable to secure confirmatory evidence on a more direct quantitative basis. To this end we ran a third series of experiments. Since in series 2 the skimmed milk portion was without effect, we decided to use only the butter fat from the various milks as the vitamin D supplements to the rations. The feeding of butter fat as the only supplement had the advantage of eliminating the repeated standardization made desirable by possible daily variations in the vitamin D content of the whole milk.

The butter fats that were used in this third series were prepared in the same manner as those used in series 2. The butter fat additions, 108 gm. in 2 kg. of ration, were adjusted by dilution with control butter fat so that equal amounts of fat were added to each ration. The general plan of this series as well as the results are shown in table 3.

The chicks on this series of experiments had to be started before the assays on the butter fats had been completed and unfortunately, the actual potencies were not as anticipated. It was found from our prophylactic assays with rats that the irradiated milk butter fat was approximately 35 per cent more potent than calculated, whereas the yeast milk butter fat was about 74 per cent as potent. In spite of this we can conclude from the results of this experiment, table 3, taking into account the results of our rat assays, that the data of this series confirm the data obtained in our previous experiments. The irradiated milk butter fat was found to be as efficient as cod liver oil whereas the yeast milk butter fat was very inefficient. Ten rat units per 100 gm. of ration did not produce any evidence of protection.

In a fourth series of experiments we increased the level of yeast milk butter fat to 25 units per 100 gm. of basal ration in the hope that a demonstrable healing would be produced. This necessitated a high level of butter fat in the ration, viz., 12.5 per cent. However, this amount of butter fat inhibited normal growth during the early part of the experiment. We consequently analyzed the bones of one-half of the chicks in the group at 6 weeks instead of at 5 weeks which was our customary procedure.

TABLE 3

Series III. Ash in the tibia of chicks fed vitamin D from cod liver oil, irradiated milk butter fat and yeast milk butter fat

SUPPLEMENTS TO THE BASAL RATION	STEENBOCK UNITS PER 100 GM. RATION[1]	NUMBER OF CHICKS	AVERAGE WEIGHT OF CHICKS	AVERAGE WEIGHT OF ASH	AVERAGE ASH
			gm.	*gm.*	*per cent*
Untreated milk butter fat	0	14	194	0.2041	32.71
Cod liver oil	2.5	11	200	0.2417	35.86
Irradiated milk butter fat	3.4	14	241	0.3204	42.92
Irradiated milk butter fat	5.0	15	253	0.3541	45.53
Cod liver oil	5.0	15	240	0.3098	39.51
Irradiated milk butter fat	6.75	11	283	0.4390	46.21
Irradiated milk butter fat	8.4	14	271	0.4003	46.94
Irradiated milk butter fat	10.1	13	237	0.3639	46.58
Yeast milk butter fat	2.5	14	217	0.2295	34.27
Yeast milk butter fat	5.0	12	185	0.2056	34.11
Yeast milk butter fat	10.0	12	201	0.2133	33.63

[1] Unitage determined by prophylactic assays with rats.

We were interested further in studying the efficacy of irradiated cholesterol as reported by Waddell ('34). For this study a cholesterol preparation was prepared and irradiated according to his technic. Preliminary assays with rats using the 10-day line test method revealed that 0.375 mg. of the irradiated cholesterol was equal to 1 rat unit. The irradiated ergosterol was similarly assayed. Both of these preparations were dissolved in Wesson oil and the desired level was obtained when the oils were added to the extent of 2 per cent of the ration. Irradiated milk butter fat was also included in this series. The results are included in table 4.

From the data presented it is apparent that the vitamin D in irradiated milk butter fat is at least ten times more effective than the vitamin D in yeast milk butter fat. Irradiated cholesterol was found to be equally as effective as irradiated milk butter fat and approximately twenty times more effective than irradiated ergosterol. We have therefore confirmed the relative high efficiency of irradiated cholesterol for the chick as reported by Waddell.

TABLE 4

Series IV. Ash in the tibia of chicks fed vitamin D from irradiated milk butter fat, 'yeast milk' butter fat, irradiated cholesterol and irradiated ergosterol

SUPPLEMENTS TO BASAL RATION	STEENBOCK UNITS PER 100 GM. RATION[2]	NUMBER OF CHICKS	AVERAGE WEIGHT OF CHICKS	AVERAGE WEIGHT OF ASH	AVERAGE ASH
			gm.	*gm.*	*per cent*
No additions	0	14	185	0.1764	33.47
Irradiated cholesterol	2.5	15	234	0.2616	40.70
Irradiated milk butter fat	3.4	15	247	0.2659	42.92
Irradiated cholesterol	5.0	12	286	0.3987	46.49
Yeast milk butter fat	25	7	184	0.2003	40.27
Yeast milk butter fat[1]	25	7	233	0.2761	36.21
Irradiated ergosterol	50	14	260	0.2983	41.40

[1] Continued on experiment for 6 weeks instead of 5.

[2] Unitage determined by prophylactic assays with rats.

DISCUSSION

The results that we have presented are for the most part not in agreement with the clinical reports that have appeared in the literature. This apparent lack of agreement may or may not be real. The efficacy ratio of cod liver oil to irradiated ergosterol for the chick has been shown to be approximately 1:20 and for the infant, 1:4 (Hess and Lewis, '33). Obviously the chick is the more sensitive of the two in regard to its differential response to vitamin D from these two sources. Since our results revealed that the efficacy ratio of irradiated milk to yeast milk is approximately 1:10, and since from the above the chick is approximately five times as sensitive to a unit of vitamin D from cod liver oil and irradiated ergosterol, it is very probable that a twofold difference in effectiveness of

the two milks is the most that could be expected for the infant. Hess and Lewis ('33) have reported this ratio of effectiveness of the two milks, although most investigators are agreed that the two milks are of equal effectiveness. From our experience with biological determinations of this nature we have become more and more impressed with the difficulties associated with the accurate determination of differences in vitamin D activity. These difficulties obviously tend, out of circumstance, to be magnified in clinical studies.

In our experiments we have been unable to confirm the reported high efficiency of yeast milk and irradiated milk as contrasted with cod liver oil or irradiated ergosterol. Nor have we obtained any indication that the presence of the constituents of milk as a vehicle for the vitamin D of either cod liver oil or irradiated ergosterol influenced either utilization or effectiveness. In series II we investigated the possibility that the skimmed milk fraction might have an influence on the antirachitic activity of these milks. Our results revealed that the skimmed milk fraction was without effect and furthermore that the differences in regard to the effectiveness of the two milks observed in our first series could be assigned entirely to the butter fat fraction.

Our results therefore indicate that the quantitative relation which has been found to exist in the response of the chick and the infant to cod liver oil and irradiated ergosterol does not exist with vitamin D milks. Our data although obtained with the chick reveal the necessity for more clinical studies of a quantitative nature concerning the antirachitic effectiveness of vitamin D milks in relation to each other and to other antirachitic agents.

SUMMARY

Briefly, the results of our experiments show that for the chick and per unit of vitamin D:

1. Irradiated milk, cod liver oil, and irradiated cholesterol are of approximately the same order of effectiveness.

2. Yeast milk is approximately one-tenth as effective as irradiated milk. This difference was confined to the respective butter fat fractions and was not influenced by the skimmed milk fraction.

3. The constituents of milk as a vehicle for vitamin D do not influence its effectiveness.

4. Our experiments give no support to the possibility that the baby chick could be used to greater effectiveness than the rat for ascertaining the degree of antirachitic effectiveness of different vitamins D for the human.

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STUDIES ON GROWTH

III. B AND G AVITAMINOSIS IN CECECTOMIZED RATS [1]

WENDELL H. GRIFFITH

Department of Biological Chemistry, St. Louis University School of Medicine St. Louis

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It is now well established that young rats survive the lack of vitamin G for much longer periods than those deprived of vitamin B. In complete B deficiency polyneuritic signs appear and death occurs after 20 to 50 days, whereas in G deficiency rats may survive for 50 to 100 days or more. Since growth ceases within 5 to 10 days after being placed on either the B-free or G-free diet, the long survival of animals deprived of G becomes a matter of considerable interest. Certainly the supply of G which is available for maintenance over such a period is not available for growth at the start of the period. This contrast in survival periods in B and G deficiency is probably related to the fact that the tissues of rats are not as easily depleted of vitamin G as of vitamin B (Graham and Griffith, '32). Vitamin G is present in the tissues of rats showing complete growth failure on a G-free diet (unpublished data). It may be that this vitamin is a tissue constituent which cannot be removed or these rats may have an unrecognized source of G which is adequate for maintenance but not for growth. The following report represents the first part of an investigation of the occurrence and function of vitamin G in the animal body and deals with the possibility of the absorption and utilization in the rat of vitamin G which may have been synthesized by microorganisms in the cecum.

[1] We wish to thank Mr. M. Sheppeck and Mr. E. E. Nixon for their assistance in this investigation.

The synthesis of vitamins B and G by the intestinal flora has been demonstrated and the necessity of preventing coprophagy has been recognized by numerous investigators. Recently, Guerrant, Dutcher and Tomey ('35) showed that the effects of coprophagy were much more marked if the carbohydrate of the diet consisted of dextrinized cornstarch rather than raw cornstarch, sucrose, lactose or glucose. An earlier paper (Bechdel, Honeywell, Dutcher and Knutsen, '28) demonstrated that appreciable amounts of vitamin B are synthesized by bacteria in the rumen of the cow and are absorbed from some portion of the alimentary canal. The early appearance of fatal deficiency signs on a B-free diet demonstrates that such a source of B is not available in the rat. It seemed that the question of an intestinal source of G in the absence of coprophagy might be answered by a study of the G requirement of young rats in which cecectomy had been performed. The cecum of the rat is relatively large and would appear to be a suitable incubator for vitamin-synthesizing organisms. With this in mind over 200 rats were cecectomized and their growth studied on various diets. After the completion of this work the paper of Guerrant, Dutcher and Tomey ('35) appeared in which a report was made of the effect of cecectomy in two rats. Their data indicated that the vitamin potency of the feces of an operated animal had been greatly reduced. In our experiments cecectomy was performed for a different purpose; i.e., to determine whether the rat regularly received significant amounts of vitamin G from the intact cecum.

EXPERIMENTAL

Young male albino rats were transferred to the experimental diet after attaining a weight of 49 to 51 gm. Such rats averaged 26 days of age. They were kept in single raised cages to prevent coprophagy. A record was made of the food consumption and of the rate of growth.

If cecectomy was performed the rats were operated 1 day after weaning. Recovery was rapid and these rats averaged 50 gm. in weight when 28 days of age.

The basal diet was the Evans and Burr mixture of purified casein, 25; sucrose, 75; and salt mixture, 4. In some experiments raw cornstarch replaced sucrose. Each rat received 0.2 cc. of fat daily, cod liver oil and lard being given on alternate days. Vitamin G was supplied in 0.4 gm. of autoclaved hog liver. Fuller's earth adsorbates, prepared from alcoholic extracts of rice bran or of wheat embryo, served as the vitamin B supplement. The combination of the vitamin B and G supplements permitted normal growth, thus demonstrating the presence of all of the necessary water soluble growth factors. In certain cases dried brewer's yeast was used as a source of the vitamins of the B complex.

THE EFFECT OF CECECTOMY ON GROWTH ON AN ADEQUATE DIET

The cecum is a dispensable organ as far as growth on the stock diet for a 100-day period is concerned. The average weight of twenty-four stock control male rats at the end of the 100-day experimental period was 284 gm. The average weight of thirty-nine cecectomized rats for the same period was 273 gm. The operated rats appeared normal in every respect and there was no evident physical difference in the feces of the two groups.

Twelve additional rats were cecectomized and fed the sucrose basal diet supplemented with fat and 0.5 gm. of dried brewer's yeast daily. This group averaged 211 gm. in weight after 50 days, an average daily increment of 3.2 gm. The absence of scurvy in this group was interesting because it was evident that freedom from scurvy in rats was independent of the cecum. The bulk of evidence at present favors the conclusion that rats synthesize vitamin C (Parsons and Hutton, '24). We are not aware of any experiments which exclude the possibility that rats may be supplied with this vitamin from intestinal microorganisms. The results with the above group rule out the cecum as a possible source of C and strengthen the belief that C is a metabolic product in the rat.

THE EFFECT OF CECECTOMY IN COMPLETE B DEFICIENCY

Fatal polyneuritis was produced by feeding the basal diet and the daily supplement of 0.2 cc. of fat and 0.4 gm. of autoclaved liver. The average period before polyneuritis appeared in forty-four unoperated rats on the sucrose basal diet was 42 days. The period was shorter on the starch basal diet. In a group of fourteen cecectomized rats on the latter diet, three died before polyneuritic symptoms were observed, deaths occurring on the twenty-third, twenty-eighth and twenty-ninth days. The remaining eleven showed the usual signs after 16 to 38 days, with an average period of 29 days. In a control group of fourteen unoperated rats on the same diet, two died before signs appeared, on the twenty-seventh and twenty-eighth days, respectively. The remaining twelve developed polyneuritis within 25 to 42 days, with an average period of 35 days. Although polyneuritis and death occurred a few days earlier in the case of the operated rats, it is not believed that the difference between the two groups indicated any appreciable absorption of vitamin B from the intact ceca of the unoperated rats.

THE EFFECT OF CECECTOMY IN COMPLETE G DEFICIENCY

G deficiency was produced by feeding the basal diet and the daily supplement of 0.2 cc. of fat and the fuller's earth adsorbate from either wheat germ or rice bran extract. The daily supplement of these adsorbates was adequate for good growth if autoclaved liver was also fed. The average weights of twelve cecectomized and thirteen unoperated rats after 70 days on the G-deficient sucrose diet were 50 gm. and 52 gm., respectively. The average weights of fourteen cecectomized and fourteen unoperated rats after 60 days on the G-deficient starch diet were 53 gm. and 46 gm., respectively. These results showed definitely that survival on G-free diets was not related to a cecal supply of vitamin G, since the cecectomized rats were in no way inferior to the unoperated controls.

Complete growth failure on a diet containing adequate supplements of vitamins A, B and D has been considered evidence

of the absence of G from the food mixture since the addition of autoclaved liver permitted normal growth. The signs of G-deficiency in young rats have been variously characterized by different investigators. Many believe that multiple factors are involved, particularly with respect to the types of dermatitis or pellagra-like symptoms which appear (Chick, Copping and Edgar, '35; György, '35). In our laboratory these symptoms have been observed to depend in part upon the carbohydrate of the diet. Young rats on the starch basal diet have never developed the brownish-red staining of the fur which is characteristic of those on the sucrose basal diet, thus confirming the work of Hogan and Richardson ('34). Evidently starch supplies a vitamin or some other dietary essential which prevents hemorrhage and the resulting staining of the fur. It may be possible that vitamin G, alone, is involved and that the amount required for growth is considerably more than that required for 'maintenance.' It appears doubtful, however, that vitamin G could be responsible for such a difference in the appearance of the two groups of rats and yet not affect the growth or survival of the groups. It is believed, therefore, that the picture of G deficiency in rats on the starch basal diet is more accurate than that observed on the sucrose basal diet. This picture is characterized by complete growth failure within 10 days; by inflammation and watering of the eyes within 10 to 20 days, followed by a sticking together of the eyelids; and, by survival for 50 days or more. Soreness of the skin on the face, around the ears, or inside the forepaws has rarely occurred. Of sixty-four rats surviving after 50 days on the G-deficient diet and showing complete growth failure only thirteen developed partial baldness. The eyes of twenty-six of these rats were examined at autopsy for evidence of cataract. Opacity of the lens was found in only two of these animals (Day, Langston and O'Brien, '31).

THE EFFECT OF CECECTOMY IN RATS ON DIETS LOW IN VITAMIN G

Although significant differences were not observed between the cecectomized and control rats in either B or G avitaminosis, it was thought that the effect of cecectomy might be

evident in more nearly normal animals, i.e., those receiving some B and G. For this purpose the sucrose basal diet was supplemented with cod liver oil, an adequate fuller's earth adsorbate of vitamin B and 50 mg. of autoclaved liver. The liver supplement prevented signs of G deficiency but permitted only subnormal growth. After 60 days, eleven cecectomized and twelve unoperated rats gained an average of 53 gm. and 58 gm., respectively.

In a second series both the sucrose basal and the starch basal diets were used and some of the rats were kept in cages

TABLE 1

The effect of cecectomy on the rate of growth of young male rats during a 60-day period on a low B and G diet[1]

SERIES	NUMBER OF RATS	AVERAGE GAIN IN WEIGHT	AVERAGE FOOD INTAKE[2]	REMARKS
		gm.	*gm.*	
190	14	38	263	Unoperated, sucrose basal, cages raised
200	14	34	251	Cecectomized, sucrose basal, cages raised
210	14	59	315	Unoperated, sucrose basal, cages on shavings
230	14	56	289	Cecectomized, sucrose basal, cages on shavings
500	28	36	271	Unoperated, starch basal, cages raised
510	29	29	253	Cecectomized, starch basal, cages raised
490	14	58	307	Unoperated, starch basal, cages on shavings
290	14	49	299	Cecectomized, starch basal, cages on shavings

[1] Basal diet supplemented with 100 mg. dried brewer's yeast daily.

[2] Total food consumed exclusive of fat supplement.

resting on shavings so that coprophagy was possible. Vitamins B and G were supplied in 100 mg. of dried brewer's yeast daily. With this supplement neither B nor G deficiency symptoms appeared but growth was subnormal. Vitamin G was the limiting factor in this yeast. The results of these experiments are shown in table 1. In every case the average gain in weight of the cecectomized rats was less than that of the unoperated controls. The differences, however, were hardly great enough to permit the conclusion that vitamin G was available in significant amounts from the intact cecum. In these experiments growth on the cornstarch basal diet was approximately the same as that on the sucrose basal diet. It

is noteworthy that coprophagy was of benefit to the operated as well as to the control rats. Vitamins of the B complex were present, therefore, in the intestinal contents of the cecectomized animals. It was evident, however, that this additional vitamin supplement was not available except through coprophagy.

DISCUSSION

The optimum requirement of vitamins B and G has been shown to be related to the composition of the basal diet by numerous investigators. These associated factors have been enumerated and discussed recently by Guerrant and Dutcher ('34) who have suggested that the nature of the food mixture may affect the vitamin requirement by influencing the microorganisms of the digestive tract. The experiments reported in this paper were performed in the hope that cecectomy might largely remove any effect of the intestinal flora and permit a more accurate determination of the metabolic need for vitamin G particularly. The experiments have failed to demonstrate a significant difference in the development of symptoms of either B or G deficiency in cecectomized and unoperated control rats. The synthesis of B or G by cecal microorganisms does not result in utilization of these substances by the rat if coprophagy is prevented. The long survival of rats on G-deficient diets and the difficulty in depleting the tissues of these rats of vitamin G are apparently not related to the presence of an intact cecum.

It is possible that there is utilization of vitamins produced by the flora of other portions of the intestinal tract. Indeed these experiments demonstrated that coprophagy benefited the operated rats. To what extent the large intestine compensated for the cecectomy cannot be stated definitely. There was some evidence that the colon undergoes change after the removal of the cecum. The intestinal tracts of operated animals were studied by the department of pathology for which we are indebted to Dr. W. D. Collier. Briefly, it was found that following cecectomy there was a marked dilatation of the first portion of the colon with a thinning and outpouching of

the wall. The fecal contents were fluid and of the consistency of the usual cecal material. The semi-solid fecal masses were formed farther down in the distal portion of the colon than is commonly the case.

CONCLUSIONS

1. Young rats grow normally for at least 100 days after removal of the cecum.

2. If vitamins B, C or G are synthesized by cecal microorganisms, they are not utilized by the rat except by coprophagy.

3. The prolonged survival of young rats on G-deficient diets is not due to a cecal supply of vitamin G.

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STUDIES ON GROWTH

IV. THE VITAMIN B AND G CONTENT OF THE BODY TISSUES OF NORMAL AND EXPERIMENTAL RATS

WENDELL H. GRIFFITH

Department of Biological Chemistry, St. Louis University School of Medicine, St. Louis

THREE FIGURES

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In earlier experiments Graham and Griffith ('32)[1] found that adult rats were readily depleted of most of the vitamin B present in the tissues whereas a corresponding loss of vitamin G did not occur. Similar experiments have been performed with young rats in order to determine to what extent vitamin G can be removed from tissues during the prolonged survival period characteristic of rats on G-deficient diets. For this purpose the tissues of rats showing complete growth failure and other signs of G avitaminosis were fed as the vitamin G supplement to rats maintained on a diet lacking in G. For comparison, such tissues were also used as a vitamin B supplement. In addition, the vitamin B and G supplementing effect of the tissues of normal and of polyneuritic rats were investigated.

EXPERIMENTAL

Young male albino rats, 49 to 51 gm. in weight and averaging 26 days of age, were used. The rats were kept in individual raised cages with bottoms of wire mesh to prevent coprophagy. The experimental diet was the Evans and Burr

[1] In this paper, line 24 on page 696 should read "more rapidly on diets low in B_1 than on diets low in B_2."

mixture of purified casein, 25; sucrose, 75; and salt mixture, 4. Two-tenths cubic centimeter of fat was fed daily, cod liver oil and lard being used on alternate days.

Rat tissues which were used as supplements were prepared in the following way. The animals were decapitated and skinned. The paws, stomach and intestines were discarded. The carcasses were than hashed in a grinder, dried in a warm air dryer at 50°C. and pulverized. In this manner, dried tissues were obtained from the carcasses of seven groups of rats.

Tissue I (6.0 gm. of dried residue per rat) represented normal rat tissue and was prepared from 122 rats averaging 50 gm. in weight. These rats were similar to those used in the feeding experiments.

Tissue II (6.8 gm. of dried residue per rat) represented the tissues of rats with B deficiency. Forty-three rats were fed the basal mixture supplemented with 0.4 gm. of autoclaved hog liver daily. This supplement was adequate for normal growth if vitamin B was also present. The rats were killed when polyneuritic symptoms appeared. The average interval before the paralysis became evident was 45 days and the average weight of the polyneuritic rats was 45 gm.

Tissue III (7.0 gm. of dried residue per rat) represented the tissues of rats with G deficiency. Twenty-seven rats were fed the basal mixture supplemented with 75 mg. of a fuller's earth adsorbate of an alcoholic extract of wheat germ. This vitamin B supplement was adequate for normal growth if autoclaved liver was used as a source of vitamin G. These rats were killed after 40 days and averaged 47 gm. in weight at the end of the experimental period.

Tissue IV (5.4 gm. of dried residue per rat) was the same as tissue III except that the rats were given only 18 mg. instead of 75 mg. of the fuller's earth preparation of vitamin B. This was done in order to minimize the possible effect of any traces of G in the activated earth. These rats, therefore, received a supplement very low in B and lacking in G. The twenty-seven rats were killed after 40 days and averaged 42 gm. in weight at the end of the experimental period.

Tissues V (7.8 gm. of dried residue per rat) and VI (6.4 gm. of dried residue per rat) were also similar to tissue III. Twenty-eight rats were fed the basal mixture supplemented with the activated fuller's earth. Fourteen of these rats were killed after 25 days and averaged 50 gm. in weight (tissue V). The remaining fourteen were killed after 46 days and averaged 45 gm. in weight (tissue VI).

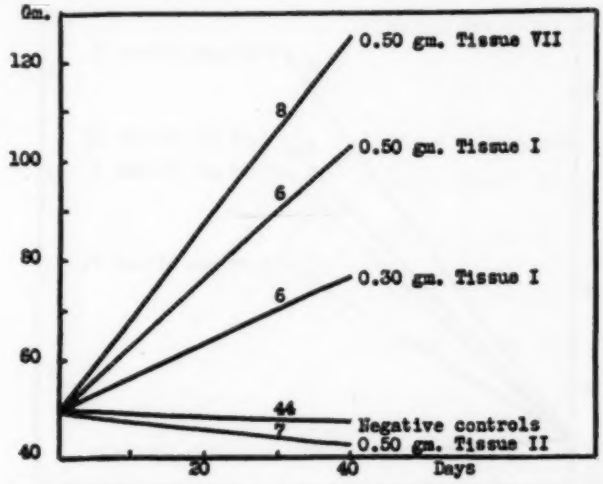

Fig. 1 The average increase in weight in a 40-day period of rats on a B-free ration supplemented with dried rat tissue as a source of vitamin B. The figure on each line shows the number of rats in the group.

Tissue VII (8.9 gm. dried residue per rat) represented the tissues of rats which had survived 100 days on the basal mixture supplemented with the fuller's earth preparation of vitamin B. The forty-five rats averaged 58 gm. in weight and showed varying degrees of hair loss. The eyes of thirty-two of these animals were examined for cataract with negative results.

The dried tissue was mixed with small amounts of the basal diet and measured portions were fed daily. No difficulty was experienced through refusal of rats to eat such supplements.

The vitamin B potency was determined by feeding the tissue preparation to rats receiving the basal mixture, fat and vitamin G in the form of autoclaved hog liver. The vitamin G potency was determined by feeding the tissue to rats receiving the basal mixture, fat and vitamin B. The latter supplement was a previously tested activated fuller's earth adsorbate of an 80 per cent alcoholic extract of wheat germ.

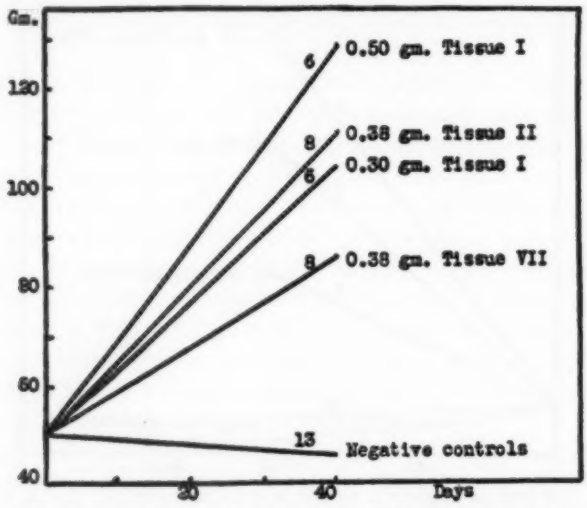

Fig. 2 The average increase in weight in a 40-day period of rats on a G-free ration supplemented with dried rat tissue as a source of vitamin G. The figure on each line shows the number of rats in the group.

The quantities of normal tissue (tissue I) which permitted definite growth as B and G supplements were determined first. Similar amounts of the experimental tissues were then used. No attempt was made to compare accurately the vitamin potency of the experimental tissues with that of the normal tissue because the weight of dried residue per rat differed with each of the seven preparations. These differences were due, probably, to the varying proportions of skeletal tissue to soft vitamin-containing tissue in the rats at the end of the

experimental periods. Therefore, the weight equivalents which were used as supplements actually represented unequal quantities of the soft tissues such as muscle or liver tissue. The amount of dried tissue required as a daily supplement for normal growth was not found because of the considerable quantity of material necessary for such determinations.

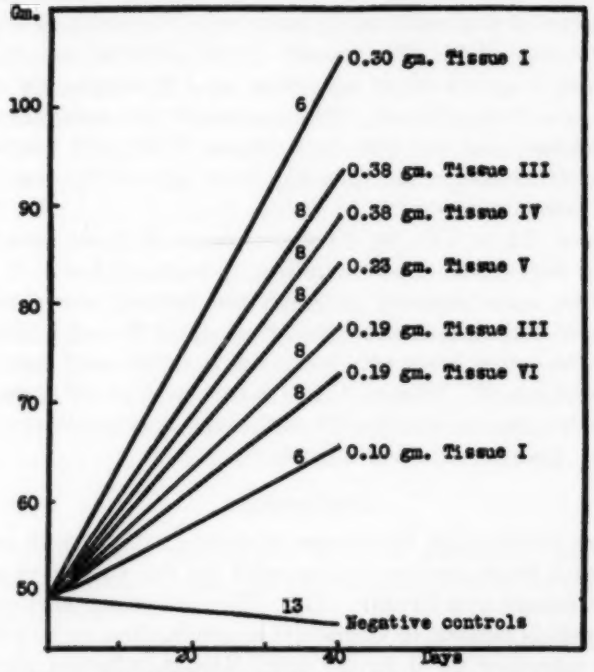

Fig. 3 The average increase in weight in a 40-day period of rats on a G-free ration supplemented with dried rat tissue as a source of vitamin G. The figure on each line shows the number of rats in the group.

Figure 1 shows the effect of feeding tissues I, II and VII as the vitamin B supplement. It is evident that vitamin B was present in the tissues of normal rats (tissue I) and of rats maintained on a food mixture lacking in G but containing B (tissue VII). However, the tissues of polyneuritic rats

(tissue II) were completely deficient in vitamin B since the rats fed tissue II as a vitamin B supplement failed to grow and developed polyneuritis as did the negative controls.

Figure 2 shows the growth which resulted when these same tissues were used as the vitamin G supplement. As was found in our earlier experiments the tissues of normal rats (tissue I) were richer in G than in B. Three-tenths gram of this tissue as a source of G permitted the same rate of growth as 0.5 gm. as the source of B. The tissues of polyneuritic rats (tissue II) which were devoid of any effect as a B supplement were potent as a G supplement. The tissues of rats maintained on a G deficient diet for 100 days (tissue VII) still contained vitamin G although the quantity was apparently less than that in normal tissues.

Tissues III to VI (fig. 3) were prepared from rats with varying degrees of G avitaminosis. Tissues III and IV had about the same potency although the former was obtained from rats maintained on a diet adequate in B and lacking in G and the latter from rats receiving a supplement low in B and lacking in G. Tissues V and VI were obtained from rats fed the G-deficient diet for 25 and 46 days, respectively. All of these tissues contained vitamin G.

DISCUSSION

These results with the tissues of young rats were in agreement with those previously reported for the tissues of adult rats (Graham and Griffith, '32). The prolonged survival of rats on diets lacking in vitamin G appears, then, to be related to the retention of G in the body tissues, whereas no such retention occurs in the case of vitamin B. Experiments are in progress to determine to what extent vitamin G may be stored as excess G and whether other tissues than the liver are involved.

The difficulty in depleting tissues of vitamin G might be explained by the presence of a small amount of this vitamin in the food or by the utilization of G synthesized by micro-organisms in the alimentary tract and absorbed therefrom

or by efficient use of the original vitamin of the tissues so that minimum waste or loss occurs. The first explanation was not considered probable because some growth would have resulted in rats which were fed the G-deficient diet if the food supplied the G actually found in the tissues of these rats. Growth failure always occurred on the G-deficient ration. The second explanation likewise appeared improbable because it has been shown that the cecum, at least, does not serve as a source of vitamin G (Griffith, '35). The third explanation appeared more plausible and it is suggested that vitamin G may be a tissue constituent which cannot be readily removed. If it is true that vitamin G is a component of certain body tissues, growth would require increasing amounts of the vitamin in the food. Booher, Blodgett and Page ('34) have observed that there is a close correlation between the requirements for vitamin G and the body weights of growing animals. The decrease in food consumption and resulting loss of weight occurring in adult rats which have been placed on a G-deficient diet indicates that vitamin G may be necessary as an appetite stimulant as well as a tissue constituent. The influence of G on the consumption and utilization of food has previously been emphasized (Graham and Griffith, '33).

CONCLUSIONS

1. Young rats maintained on a B deficient ration were readily depleted of most of the vitamin B originally present in the tissues.

2. A corresponding loss of vitamin G did not occur in young rats on a G-deficient ration.

3. The prolonged survival of rats on a G-deficient ration is related to the retention of tissue vitamin G.

4. It is suggested that vitamin G may function in part as a tissue constituent.

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VARIABILITY OF VITAMIN D RESPONSE WITH TEMPERATURE OF ENVIRONMENT [1]

D. TOURTELLOTTE AND W. E. BACON

General Foods Corporation, Battle Creek, Michigan

TWO FIGURES

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Anyone who has followed the vitamin D assay closely for any extended period of time has certainly encountered all manner of variation, some of which can be significantly related to definite factors and others which remain obscure. Probably the most exhaustive study of the vitamin D determination has been reported by a group of workers from the Lister Institute and the National Institute for Medical Research, London, Hume et al. ('32), Bourdillon et al. ('32). They have been extremely careful in their studies of the preparations which have served as the International vitamin D standards. In this connection they have reported the errors involved in the different vitamin D assay procedures.

In addition to the errors inherent in the methods used they also found a variation in the sensitivity of the stock of rats to rachitogenesis from time to time which could not be related to the vitamin D. Quoting from Hume ('32):

Whether such a variation in the response of the experimental animals at different times is due to variation in the animals' reserves of vitamin D or to variation in the rickets-producing power of any of the constituents of the basal diet or to some other unrecognized cause, is at present not known, but it is one of the greatest causes of difficulty in the biological estimation of vitamin D.

[1] Presented before the American Institute of Nutrition, April 10, 1935, at Detroit.

In a test of the stability of the International vitamin D preparation extending over 21 months, Bourdillon encountered maximum fluctuations in excess of 300 per cent in the apparent potency. From their results, however, they concluded that there was no appreciable deterioration in vitamin D potency and that the major variations were due to changes in the general sensitivity of the stock.

Bills and co-workers ('31) have reported a critical study of the line test results accumulated from their experience with routine laboratory assays of vitamin D-containing materials. Bills has treated the errors within any assay group but does not mention a variation in sensitivity of the test animals from time to time. In a private communication Doctor Bills has told us that he has not encountered this difficulty under his present conditions for conducting vitamin D assays.

We have standardized so far as possible in the conduct of our animal colony, for ruling out the variations due to dietary differences. We purchase a year's supply of grains and have established a constant source of supply for other constituents in the rachitogenic diet. The rats used in our colony are the McCollum inbred strain.

We have attempted to take care of the matter of environmental temperature by placing a thermostatic control on our heating system. However, this does not take care of the heat drawn from other parts of the building by our ventilating fan nor does it take care of excessive summer temperatures. We have no means of regulating humidity although it must certainly be a factor in the control of laboratory animal quarters. In this connection several laboratories in this country have been equipped for year round control of temperature and humidity.

We use the McCollum line test procedure as modified by Bills. The 5-day assay procedure as introduced by Bills possesses the advantage of speed and reliability over other existing vitamin D methods. We use the McCollum rachitogenic diet no. 3143. The autopsy and line test procedure are conducted by the usual technic. The tibia healing is graded by

direct observation of the stained bone according to the scale devised by Bills. Since the four grades of healing bear no simple numerical relation to each other he has developed a graphical relationship of healing to potency which is another feature in the assay technic and which gives an exact potency factor for any degree of healing within the sensitive range of the test.

We have used the 5-day assay procedure since the latter part of 1931. Since 1933 and until the U.S.P. vitamin D standard became available we used cod liver oil as a control standard for every vitamin D assay which we made. Our auxiliary standard was checked against the U.S.P. vitamin D standard when it became available and fortunately they agreed very closely. Thus since early in 1933 until the present date we have controlled all vitamin D assays with either the auxilliary standard or with the U.S.P. standard containinf 95 U.S.P. units per gram.

Although we have been aware of a fluctuation in the sensitivity of our test animals prior to the use of standardized preparations as control for our assays we had no means of knowing its magnitude. The accumulation of results from the assay of reference standards showed an apparent fluctuation in potency of approximately 400 per cent. Our first notion was that the fluctuation was in some way a seasonal problem since the results showed their greatest depression during the summer months. Fortunately we had kept daily 24-hour records of the laboratory temperature and when our potency values were correlated with these we found that the greatest depressions in apparent potency coincided with high laboratory temperatures during the rachitogenic and test periods (fig. 1). A correlation diagram has been constructed (fig. 2) relating the apparent potency with the mean daily temperature of the combined rachitogenic and test periods (26 days) and it shows that laboratory temperatures of 80°F. and above give rise to considerable variation in the apparent potency of standard reference materials.

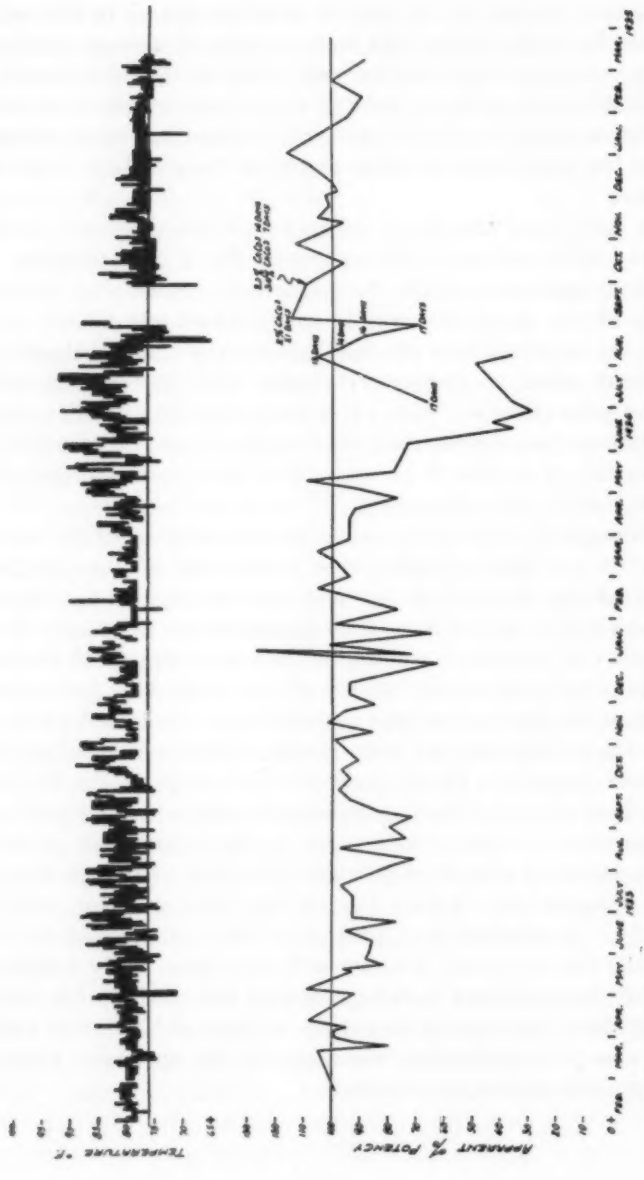

Fig. 1 Variation in apparent potency of vitamin D reference standard together with temperature variation over 2-year period.

To show that the fluctuation during periods of high laboratory temperature is due to an increased severity of rachitogenesis we have been able to conduct satisfactory vitamin D assays on rachitic animals after only 11 to 13 days on the rickets-producing diet, as against the regular rachitogenic period of 21 days. Examination of these 11- to 13-day

Fig. 2 Correlation diagram, relating mean temperature of rachitogenic and test periods with apparent assay potency of reference vitamin D standard.

rachitic animals showed a satisfactory grade of line test rickets.

We have also been able to reduce the severity of rickets produced in the regular rachitogenic period by reducing the Ca : P ratio of the diet. The McCollum diet no. 3143 regularly contains 3 per cent $CaCO_3$ and a Ca : P ratio of about

5:1. By reducing the $CaCO_3$ to 2 per cent good line test rickets have been produced in 21 days during these periods of uncontrollably high temperatures.

In conclusion, we have found that to a certain extent the variation in the sensitivity of the rachitic test animals is due to fluctuating laboratory temperatures during the rachitogenic and test periods. An increased severity of rachitogenesis results in vitamin D test animals when they are subjected to laboratory temperatures of 80°F. or above. We believe that it is fully as desirable to define a standard rachitic test animal in terms of response to a definite amount of a standard vitamin D preparation as it is to prescribe the exact conditions for rachitogenesis and methods for administering the test preparations.

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EDITORIAL REVIEW

THE ABSORPTION AND UTILIZATION OF CARBOHYDRATES

H. B. PIERCE

Department of Vital Economics, University of Rochester, Rochester, N. Y.

Carbohydrates have three primary functions in nutrition, 1) to furnish energy and heat, 2) to build glycogen as a reserve store for energy and 3) to make fat. In addition, carbohydrates are used to fulfill many other minor needs of the body. They function in the synthesis of certain cerebrosides and glyco-proteins and in stimulating the production and flow of insulin, intestinal juice and possibly bile. They protect the liver against damage resulting from chloroform poisoning. Evidence is available showing that certain carbohydrates cause marked changes in the intestinal flora. The sparing action of carbohydrate on protein metabolism is well recognized, and in bacterial culture media, the addition of sugars tends to prevent or limit the formation of putrefactive products. Another role is their entrance into certain phases of mineral metabolism.

As this subject is one of tremendous scope, the present review will be limited as far as possible to considerations involving normal organisms and the more common carbohydrates. Even with these limitations, the great volume of literature necessitates the omission of many references.

ABSORPTION OF CARBOHYDRATES FROM THE ALIMENTARY TRACT

The absorption of carbohydrates from the gastro-intestinal tract of man and animal has been of interest to many investigators. In this section, their absorption from the stomach and

small intestine will be reviewed. The experimental procedures used are not at all similar, a fact which undoubtedly accounts for the variable results reported in the literature.

Absorption from the stomach. Data have been obtained, a) by use of animals with gastric fistulas, b) by tying the stomach at the pylorus and recovering the sugar introduced at a later hour, c) by determining the sugar content of blood flowing from the stomach, and d) by measuring differential changes in the concentration of a sugar and non-absorbable substance mixed together and recovered after the period of absorption. We have found the last method unsatisfactory in determining sugar absorption from the intestine and Maddock, Trimble and Carey ('33) have commented on the complexity of the computations following employment of this technic.

A review of the literature concerning the gastric absorption of sugars having been presented recently by Maddock, Trimble and Carey ('33) only brief mention of results will be presented here. It is the contention of some workers that glucose is absorbed from the stomach, particularly if in alcoholic solution, but others have proved that no glucose or sucrose is absorbed under these circumstances. Roth and Strauss (1889) in discussing their results (p. 178) stated Der Magen ist also ein Organ der Sekretion, welches zur Resorption ungeeignet ist."

Experiments performed by Macleod, Magee and Purves ('30) on urethanized rats led them to conclude that little or no glucose was absorbed from stomachs ligated at the pylorus. Maddock, Trimble and Carey ('33) reached the same conclusion as they found no increase in the sugar content of blood in gastric veins of dogs after placing glucose in their stomachs. An average of 99 per cent of the glucose introduced was recovered after absorption periods of 1 to 2 hours.

The widely accepted view of today is that the amount of sugar absorbed from the stomach under conditions of normal digestion is small and perhaps insignificant.

Absorption from the small intestine. Although some of the procedures outlined under 'absorption of the stomach' have

been used in studying intestinal absorption of carbohydrates, the majority of the experiments have been carried out by one of three methods, a) by use of isolated segments in anesthetized animals or Thiry or modified Thiry loops in unanesthetized animals, b) by use of either all or the greater part of the gastro-intestinal tract in anesthetized or unanesthetized animals and c) by determining the sugar content of the blood during absorption. Here again differences in technic have made it difficult to compare and correlate data from various laboratories. Anesthesia, the section of the intestine used, the condition and age of the animal, and the effect of the stomach or its secretions on the sugar solution have not been taken into consideration in the evaluation of many data. Although Cori ('31) stated that the blood sugar curve was not a measure of the rate of absorption, Magee and Reid ('31) found the percentage increases in blood sugar curves were in accord with absorption coefficients (see p. 692, this text) obtained in both anesthetized and unanesthetized animals. While this conclusion may apply to their particular experiments, it has no standing as a general proposition, as is shown further on in this paper.

For the sake of clarity, the subject of intestinal absorption of carbohydrates will be divided into five parts; 1) absorption rates of different sugars, 2) uniformity of absorption rates over periods of time, 3) effect of concentration of sugar solutions on absorption rates, 4) effect of the section of the tract used on absorption rates and 5) effect of the stomach and its secretions on absorption rates.

1. Absorption rates of different sugars. It seems natural to assume that there is a relationship between the rates of absorption and utilization of carbohydrates. Early results were obtained by use of isolated segments of various sections of the intestine of anesthetized or unanesthetized animals.

From data presented in papers by Nagano ('02 a and b), Röhmann and Nagano ('03), Cori ('25), Verzár ('35) and Feyder and Pierce ('35) it may be concluded that the disaccharides are absorbed more slowly from the intestine than are

the monosaccharides, with the exception of fructose. Glucose and galactose are absorbed at approximately the same rate which is considerably higher than that for fructose, while the disaccharides sucrose and maltose are removed more rapidly than lactose. In solutions containing both glucose and galactose, glucose passes through the intestinal wall more rapidly than galactose.

2. Uniformity of absorption rates over periods of time. Hédon ('00) determined the rate of absorption of glucose for 0.5 to 6.0 hours and his results revealed an initial rate of 1.25 gm. per hour while 6 hours later this had dropped to 0.75 gm. per hour.

A number of years later Cori ('25) fed rats known quantities of hexoses and pentoses by stomach tube and after definite intervals of time sacrificed the animals, recovering the unabsorbed sugar from the gastro-intestinal tract. As a measure of the amount of sugar absorbed he used a 'coefficient of absorption' defined as that amount of sugar absorbed per 100 gm. body weight per hour. The coefficient remained constant from hour to hour or until all of the sugar had disappeared from the tract. After prolonged fasting the absorption rate remained constant, but at a lower level (Cori, '31). The agreement of these coefficients among groups was less variable in Cori's data than in those of Pierce, et al. ('29), Feyder and Pierce ('35), MacKay and Bergman ('33 a and b) and Magee and Reid ('31). In our data all results were included in the average provided no abnormalities were observed during the absorption period. The greatest range for absorption coefficients was noted in results for the 1-hour period. For the sake of comparison of various coefficients of absorption of glucose (milligrams per 100 gm. body weight per hour), the following table is inserted:

	Hours		
	1	*2*	*3*
Cori ('25)	196	161	178
Cori, et al. ('29)	203	235	220
Pierce, et al. ('29)	236	229	168
Feyder and Pierce ('35)	213	181	160

Magee ('30) stated that Auchinachie, Macleod and Magee in unpublished work had, in general, confirmed Cori's results for sugar. Holtz and Schreiber ('30) also reported that the absorption of glucose took place at a constant rate. Trimble and Maddock ('34) introduced glucose solutions directly into the lumen of the duodenum of dogs and concluded that the sugar was absorbed at a uniform rate over a period of hours.

Although the findings of Cori ('25) have been confirmed by several workers just cited, others have obtained results which are not in agreement with his. Pierce, Osgood and Polansky ('29) investigating the rate of absorption of glucose from the alimentary tract of rats with the Cori ('25) technic, found there was a falling off in the absorption rate during the second and especially the third hour after feeding. Cori, Cori and Goltz ('29) pointed out that Pierce, et al., had not fed enough sugar to permit the sustenance of a uniform rate of absorption for a 3-hour period. This seemed a logical criticism in light of the data submitted. Cori et al. ('29) on the basis of new data submitted, reaffirmed the former conclusion that glucose was absorbed at a constant rate. Magee ('30) stated, "Cori's opinion may then be taken as substantially correct; but all the published results including his own, show a slight falling off in the rate as absorption proceeds."

Data submitted by Burget, Moore and Lloyd ('32, '33), Ravdin, Johnston and Morrison ('33 b), MacKay and Bergman ('33 a and b) and Feyder and Pierce ('35) failed to show a linear relationship when the amount of sugar absorbed was plotted against time. MacKay and Bergman ('33 a and b), Pierce, et al. ('29) and Feyder and Pierce ('35) noted that there was a closer agreement between the amount of glucose absorbed per unit of surface than per unit of weight, a finding disagreeing with that of Maddock, Trimble and Carey ('33). MacKay and Bergman ('33 b) in discussing the results of Trimble, Carey and Maddock ('33) suggested that the uniformity in the rate of absorption of glucose noted by the latter workers was due to the increasing amounts of glucose fed for the longer absorption periods. In replying, Maddock, Trimble and Carey ('33) selected data from their earlier paper indi-

cating that the dosage of glucose had nothing to do with the rate of its absorption.

3. Effect of concentration on absorption rates. Nagano ('02 a) and Omi ('09) using comparatively dilute solutions of several sugars, obtained results demonstrating that the total amount absorbed increased as the solution became more concentrated, although the percentage of the total absorbed decreased.

London and his associates ('08) approached the question of absorption in relation to concentration by an entirely different method. They introduced solutions of glucose ranging in concentration from 4.6 to 79.7 per cent into a fistula leading to the duodenum and later recovered the unabsorbed sugar from a fistula in the ileum. While a uniform absorption of approximately 0.4 gm. per kilo per hour prevailed from glucose solutions ranging from 11.5 to 37.5 per cent concentration, their total data showed absorption rates varying from approximately 0.2 to 0.9 gm. per kilo per hour. Although Cori ('25) concluded that the rate of absorption of hexoses was independent of the absolute amount and also of the concentration of the sugar present in the intestine, his own data (table III, p. 699) demonstrated that by increasing the amount of glucose fed per 100 gm. body weight from 0.819 to 1.459 gm., the coefficient of absorption rose from 143 to 184, a difference of 30 per cent.

In several different types of experiments, Auchinachie, Macleod and Magee ('30), Macleod, Magee and Purves ('30) and Magee and Reid ('31) obtained data showing that optimal absorption of glucose took place from 0.75 M (approximately 13 per cent) solutions of the sugar. Magee and Reid ('31) found that if the 0.75 M glucose was prepared in 0.2 per cent phosphate at pH 7.0, the absorption rate of the sugar increased by 50 per cent.

Glucose solutions of different concentrations are diluted to approximately the same percentage composition prior to their absorption from the intestine, according to Ravdin, et al. ('33 a). The solutions were administered by the Cori ('25) technic. Thus, they found that the glucose solution in

contact with the absorbing surface of the intestine had approximately the same concentration irrespective of that of the solution fed. It was believed by these authors that their failure to confirm Cori's ('25) results with respect to the effect of concentration upon absorption rate might have been due to neglect of dilution factors, introduced by the stomach, in that their solutions were introduced directly into the intestine.

Trimble, Carey and Maddock ('33) adapting Cori's technic to dogs, found no definite relationship between variations in concentration or absolute weights of glucose ingested and the rate of absorption. Trimble and Maddock ('34) reported that irrespective of the concentration of the glucose solutions used (3 to 32 per cent) the rates of absorption always averaged 0.92 gm. per kilo per hour for periods up to 3 hours, the sugar having been introduced directly into the lumen of the duodenum. Where the concentration of the glucose solution was low and the volumes of fluid introduced large, the Woodyatt type of pump was used. The use of this technic would require a consideration of possible effects of intra-intestinal pressure on absorption.

More recently, Verzár ('35) investigated the effect of concentration on the rate of absorption of several sugars from the intestine in urethanized rats. He selected a section of upper jejunum 30 cm. in length, washed it out with saline and after 1 hour introduced the sugar solution. One hour later, the animals were killed and the sugar remaining in the segment washed out and determined. Verzár concluded that the resorption of glucose and galactose from their solutions (1 to 14 per cent) was independent of their concentration, but this did not hold true for fructose, xylose, sorbose or mannose, where more sugar was absorbed with the more concentrated solutions. He believed that the absorption of glucose and galactose took place through active cell processes, while that of the other sugars studied resulted from pure diffusion.

4. *Effect of the section of intestine used on absorption rates.* It is generally conceded today that absorption from the upper part of the intestine is greater than from the lower. Röhmann and Nagano ('03) studying absorption rates of the disac-

charides from Vella fistulas of jejunum and ileum and later isolated loops of various sections of dog intestine, obtained data which revealed a more active absorption from jejunal than from ileal segments. The following values may be taken as illustrative of results obtained with 5 per cent solutions of disaccharides introduced into 30 cm. lengths and allowed to remain 1 hour.

	Grams absorbed	
	Jejunum	*Ileum*
Sucrose	2.39	0.95
Maltose	1.56	0.67
Lactose	0.75	0.26

Omi ('09) confirmed this point with 1 to 8 per cent sucrose solutions, always finding more absorption from the jejunum than from the ileum.

5. Effect of the stomach and its secretions on absorption. There can be no question that the emptying time of the stomach plays a role in affecting sugar absorption from the gut. Adam ('24) using infants, and Holtz and Schreiber ('30) using dogs, observed, by use of fluoroscopic methods, the movement of sugar solutions containing $BaSO_4$ or colloidal SiO_2 through the alimentary tract. Both saw the delayed passage of such solutions into the intestine, and the latter reported a closure of the pylorus for as long as 20 minutes in the midst of an absorption period. Macleod, Magee and Purves ('30) also Ravdin, Johnston and Morrison ('33 a) noticed that 1 to 2 hours after feeding glucose to animals, and while their stomachs still contained noteworthy quantities of sugar, little or none was found in the intestine. Macleod et al. ('30) also noted that the rate of discharge of sugar solutions through the pylorus varied inversely as their concentrations. As Cori ('31) pointed out, more work is required to determine whether the emptying time of the stomach is a limiting factor in absorption. Certainly the results cited above suggest strongly that pyloric activity must be considered in studies such as these.

ASSIMILATION OF CARBOHYDRATES

Many factors, such as endocrine disturbances, state of nutrition, age and sex of subject, fevers, pregnancy, fatigue,

activity, and temperature, may influence the assimilation of carbohydrates. Insofar as possible, the results of experiments on normal humans or animals will be reviewed and only brief summaries of some of the more important investigations can be presented.

As soon as sugar passes through the intestinal wall, it enters the blood stream and one quite naturally expects to find a rise in blood sugar levels. The rise and fall of blood sugar, during the earlier part of an absorption period at least, is dependent upon the relative rate of inflow of sugar from the intestine and the rate of its subsequent removal from the blood by the tissues. When large amounts of sugar are ingested, it is not surprising that the rate of absorption should exceed the rate at which the tissues can remove them. On the other hand, it is not clear why the blood sugar curve should fall to normal and below while the rate of absorption from the gut is still high. Apparently in the normal subject, some dormant factor awakens at a certain time after taking sugars and checks hyperglycemia.

Much of the earlier work in this field was unreliable due to the non-existence of satisfactory quantitative methods for the determination of blood sugar. Thus the assimilation limit of carbohydrates was determined by finding what amount of sugar could be fed without having any excreted in the urine, no attention being paid to blood sugar levels.

Macleod ('16) determined this 'assimilation limit' of glucose by feeding it to fasting subjects and testing the urine for sugar at frequent intervals. The majority of normal healthy individuals showed no glycosuria from a dose of 200 gm. With increasing doses up to 500 gm. more and more subjects had glycosuria. As much as 300 gm. invert sugar or sucrose has been fed to men with no evidence of urinary sugar. Field ('19) fed 100 gm. doses of various sugars to fasting adult Negroes, plotting curves for blood sugar and testing the urines for sugar at frequent intervals. With this amount of any of the sugars no glycosuria was exhibited. Glucose, maltose and mannite gave quite similar curves, the height being reached 1 hour after ingestion. The peak for the

mannite curve was not as high as for the two sugars. The rise was not so rapid with sucrose, the largest amount being present at the end of 2 hours. Lactose had practically no glycemic action. Mendel and Jones ('20) fed healthy rabbits 50 cc. of different sugar solutions varying in concentration, by stomach tube. After 3½ hours, the urine was tested for sugar and if no reducing substance was present, the amount of sugar for the next dose was increased. The smallest amount of carbohydrate which resulted in glycosuria was recorded as the 'assimilation limit' and these, in grams per kilo, were given as follows: sucrose 7.5, fructose 8.0, glucose 13.0, maltose 17.1, dextrin 16.1. These data were partially confirmed and made more complete by Ariyama and Takahasi ('29). Jones ('20) found that glucose raised blood sugar the most, dextri-maltose and dextrin following in the order given. The results with sucrose were somewhat erratic. Fructose, of the sugars used, was the only one which failed to raise blood sugar.

Folin and Berglund ('22) have reviewed additional papers dealing with the older work on sugar assimilation and have noted the many conflicting points of view expressed by numerous workers in the field. Jacobsen ('13) was one of the first to use an accurate method (Bang's micro method) in an attempt to correlate blood sugar levels with urinary sugar excretion. He used starch, glucose, protein, fat, and fat with starch in his experiments with normal and pathological subjects. Normal subjects were given either 100 gm. glucose or 167 gm. of white bread before or 2 to 3 hours after breakfast. At 15-minute intervals after the ingestion of the carbohydrates, sugar was determined in the blood and in urine samples, when obtainable. Eight of his fourteen subjects exhibited glycosuria after ingesting 100 gm. glucose, this commencing when blood sugar increased to 160–170 mg. per cent. The blood sugar level rose rapidly reaching a peak in from 15 to 45 minutes and returned to the original level some 2 to 3½ hours later. With 167 gm. white bread, seven of the fourteen subjects showed glycosuria, the blood sugars reaching approximately the same level as noted with glucose, but returning to normal more slowly. Not being willing to accept

Jacobsen's figures as the standard for normals, Hamman and Hirschman ('17) pointed out that Hopkins ('15) after feeding 100 gm. glucose to normal persons, found the blood sugar to reach maximal levels of from 110 to 156 mg. per cent, but no tests were made on the urine. Hamman and Hirschman gave fasting subjects 100 gm. glucose in lemonade after a night's fast and noted that blood sugar rose rapidly to approximately 0.15 per cent, usually within 30 minutes, and returned to the original level within 1 to 2 hours. The renal threshold for glucose in normals was found to be 0.17 to 0.18 per cent. Folin and Berglund ('22) really explained the variable results of different workers when they said that the hyperglycemia just adequate for the production of glycosuria, if produced at all, lasted for so short a time that one seldom succeeded in collecting urine samples of that particular period.

Goto and Kuno ('21) published results of a series of threshold experiments on fifty-three Japanese subjects, giving them 100 gm. of glucose in 250 cc. of water after a night's fast. Thirty-three of the fifty-three subjects excreted sugar, the highest amount being 0.8 gm. The average blood sugar level of those persons not excreting glucose was 142 mg. per cent and of those excreting sugar 160 mg. per cent. With all subjects the alimentary hyperglycemia reached a maximum 40 to 60 minutes after the test and became normal within 3 hours.

The object of the experiments of Folin and Berglund ('22) was to determine the relationship between concentration of sugar in the blood and its elimination in the urine. They used glucose, fructose, galactose, lactose, dextrin and starch as sources of carbohydrate. When 200 gm. glucose were ingested by normal persons, blood sugar did not rise above the threshold and no glucosuria was noted. None of the other carbohydrates ingested raised blood sugar to the same extent as glucose, a point which other investigators have questioned and which will be discussed later. Although Folin and Berglund found that galactose and lactose had little hyperglycemic action, they noted a marked excretion of these sugars in the urine. Blood sugar fell even when absorption of sugar from the intestine was still going on. This was believed to be

due to the removal of sugar from the blood by the tissues rather than to glycogen formation. The subsequent hypoglycemia noted was a result of a decreased need for sugar transport from one tissue to another.

A comparison of the concentrations of sugar in arterial and venous blood during active absorption of several sugars from the intestine of humans was made by Foster ('23 a). The 'glycemic response,' following ingestion of different sugars, was noted by determining the sugar content of both venous and arterial blood, and was found to be dependent upon the carbohydrate used.

In commenting upon these and other results, Foster ('23 b) said that when a large amount of sugar was ingested, it was not surprising that the rate of absorption exceeded the rate at which tissues could remove it, but it was not clear why the curve returned to normal while the rate of absorption from the gut was undoubtedly still high. A further oddity was the continued fall of blood sugar even below the original level after glucose, but not with fructose or galactose. Further, it was not clear why a second dose of glucose failed to cause the same rise in blood sugar as noted with the first. Foster believed that the ingestion of glucose and the subsequent hyperglycemia stimulated insulin production which in turn accelerated both glycogen synthesis and oxidation of sugar, this being an idea supported in whole or part by the findings of Bornstein and Holm ('22). Soskin and Allweiss ('34) and Soskin ('35) do not believe the stimulation of insulin production is the correct explanation of the hypoglycemia, but detailed results are not yet available for review. Foster suggested that the carbohydrate oxidized and from which glycogen was formed, was not ordinary α or β glucose but some substance formed when ordinary glucose was acted upon by insulin. This substance, he thought, was more readily formed from fructose, perhaps without the aid of the pancreatic hormone. As is seen, Foster's conclusions are quite different from those of Folin and Berglund ('22).

Several workers have observed that of the three common hexoses galactose produces the most marked hyperglycemia

followed by glucose and fructose. Glucose, but not fructose, given in equal quantity with galactose, reduced the hyperglycemia of galactose to a level slightly greater than that of glucose when given alone. This effect of glucose was noted by Folin and Berglund ('22) and later by Reinhold and Karr ('27).

It was found by Reinhold and Karr ('27) that galactose, maltose, sucrose, starch, lactose and fructose were effective in the order named in producing alimentary hyperglycemia in rabbits. They believed the ability of the various carbohydrates to cause hyperglycaemia varied directly with their rates of absorption and inversely with their ability to form glycogen and the ease with which they were oxidized. The tolerance for the different carbohydrates, starch excepted, was reduced by fasting. Factors stimulating the glucose-removing mechanism of the blood were discussed.

It is interesting to note that quite different results have been obtained when smaller doses of sugar were fed to fasting subjects. For example, it has been reported that after feeding 20 gm. of glucose, fructose, sucrose, lactose and galactose, the hyperglycemia was most marked with glucose and least with galactose, although a large amount of the latter was excreted in the urine. Starch (20 gm.) had no glycemic effect. Inasmuch as only single observations were made with some of the sugars, too much reliance should not be placed on the results.

Rowe ('24) selected galactose as the most satisfactory of the sugars to use for tolerance tests because small quantities were required and no digestive disturbances followed. The tolerance dose for men was found to be 30 gm. and for women 40 gm. This was explained on the basis of a difference between the galactose metabolism of man and woman.

Having noted that the native races of Natal regularly ingested huge quantities of sweets, May ('28) determined tolerance curves after giving 50 gm. glucose and found them normal. He then gave nine men 1000 gm. of pure glucose. All showed glycosuria which persisted for as long as 18 hours after the dose of sugar. The blood sugars with 1000 gm. glucose were higher than with 50 gm. in only three cases and in all

but three, blood sugar was approaching the normal level after 2 hours. Four of the nine showed only a slight rise in blood sugar after ingestion of the huge quantity, yet exhibited glycosuria.

Koehler, Rapp and Hill ('35) recently compared the glycemic responses of normal subjects after the ingestion of 80 to 120 gm. of lactose, starch and glucose. Lactose had no hyperglycemic effect, this having been noted earlier by Field ('19), Folin and Berglund ('22) and others. Lactosuria was not uncommon. The ingestion of starch and glucose resulted in rises in blood sugar of about the same magnitude, a peak being reached after 1 hour. The glucose curve fell more rapidly than the starch, also with the former, the level fell below the original shortly after the second hour, but the total hyperglycemia after starch was greater than for glucose.

A most interesting series of experiments with sixty infants 2 to 10 days of age, was reported by Greenwald and Pennell ('30). Distinct differences in curves for blood sugar levels of infants and adults were revealed following ingestion of sugars. After glucose intake, curves of adults and infants were practically parallel, except that those for the latter were considerably lower. The difference in response to various sugars was most striking, for blood sugar rose to practically the same level with infants following glucose, sucrose, lactose, or dextrimaltose, a response totally different from that of adults.

Using intravenous injection in contrast to ingestion, Wierzuchowski, et al. ('31) found when glucose, fructose and galactose were given at a rate of 2 gm. per kilo per hour to well-nourished fasting dogs that blood sugar rose tremendously with galactose 800 mg. per cent, with glucose to 300 mg. per cent and fructose 260 mg. per cent. About 10 per cent of the total amount of glucose and fructose injected was lost in the urine, while some 70 per cent of the galactose was so lost. Injections of insulin simultaneously with the three sugars mentioned, reduced the glycemic action of glucose and galactose, but had no effect on fructosemia.

It is evident from the preceding discussion that several factors are responsible for the variable effects of different

carbohydrates on blood sugar levels. The rate of digestion and absorption, as well as the rate of passage through the alimentary tract, are of importance.

UTILIZATION OF CARBOHYDRATES

A preferential utilization of various carbohydrates is shown from the embryonic to the mature state of the organism. The presence of lactose in milk, and the larger quantity in human than in cow's milk, affords an interesting point of discussion. Many papers on this subject are theoretical, yet others present evidence as to the real importance of lactose for the nourishment of the young.

It is thought by some investigators that the high lactose content of human milk may be correlated with the very much greater brain development of young human beings. There is a very rapid myelinization of the fibers of the brain occurring shortly after birth during the first 6 weeks of life, this requiring extra galactose for cerebroside (galactoside) formation. The special need of this sugar in the diet of infants, has also been ascribed by some to its power of maintaining a normal intestinal flora, non-putrefactive in type. Bergeim ('26) (see also Kline, et al., '32) found that by feeding lactose to young animals, calcium and phosphorus absorption from the intestine was increased. The favorable effect noted was thought to be due to increased lactic acid formation which resulted in a more marked acidity of the feces.

As a result of observations with some 1000 children, Jarvis ('30) recommended that extra lactose should be given all infants receiving cow's milk. Extra quantities of 10 gm. at 3-day intervals were well tolerated. Babies receiving extra lactose were found to have better resistance to infection, firmer tissues, also more freedom from constipation and other nutritional and nervous upsets than a group not receiving additional lactose.

The relationship between growth and the carbohydrate ingested has been investigated by several workers. Mitchell ('27) fed rats entirely adequate diets containing 60 per cent of one of the following carbohydrates; starch, dextrin, lactose,

sucrose or maltose. The growth of the rats was normal or above for all except lactose, while starch and dextrin produced the most rapid weight increases. The diarrhea accompanying lactose ingestion prevented its utilization. If, however, the diets contained 30 per cent or less of lactose, the balance of the carbohydrate being made up by corn starch, growth was normal. From studies of growth rates of young rats, Jarvis ('30) stated that an associate in unpublished work found that those receiving lactose as the exogeneous source of carbohydrate gained at a slower rate than sucrose fed animals (16 per cent less). There was significantly less water and fat in the tissues of the lactose than in the sucrose fed animals.

Ariyama and Takahasi ('29) fed young rats a carbohydrate-free basal diet, and supplemented it by adding equivalent weights of each of several carbohydrates so that the diet contained 30 per cent. The growth of the rats was followed for periods of 40 to 70 days, after which the weight gain in grams per day due to each carbohydrate was calculated. The gains in grams per day were as follows: maltose 1.63, fructose 1.38, starch 1.34, glucose 1.31, dextrin 1.28, lactose 1.27, sucrose 1.16, inulin 1.14, galactose 0.86 and carbohydrate-free 0.89. No explanations of the findings were offered. Koehler and Allen ('34) determined the relative nutritive values of glucose, sucrose and lactose by noting the gain in weight of rats after the sugars were added in equivalent amounts at 35 per cent levels to sub-maintenance diets. After 5 days, the continued weight gain was materially higher with glucose and sucrose than with lactose, due to the poor utilization of the last.

In the studies dealing with the relationship between growth and the carbohydrate ingested, ad libitum feeding experiments were used and conclusions drawn from them must not be interpreted as strictly correct.

Marked differences in the utilization of several simple sugars by various animal organs and tissues, including nerves, have been observed. Cori ('31) has reviewed factors influencing carbohydrate utilization by man and animal and a discussion of these will be omitted.

COMBUSTION

Many workers have measured sugar utilization by noting changes in the R.Q. following ingestion of various sugars. Higgins ('16) found the respiratory quotient rose rapidly following ingestion of levulose, sucrose, or lactose, although with lactose the rise was not so marked. With glucose and maltose, a definite rise did not occur for 20 to 30 minutes. These results have been confirmed by the data of other workers published more recently. Bornstein and Holm ('22) and Holm ('23) published the results of a number of observations obtained following the ingestion of glucose, fructose and lactose by man (dosage 100 gm.) and animal (dosage 35 gm.). The blood sugar following glucose rose immediately, although burning did not begin for some 30 to 90 minutes. The blood sugar rise following fructose or lactose was negligible, but fructose began to burn within 5 to 8 minutes and lactose some 60 minutes after ingestion. The R.Q. with fructose rose higher (frequently 1.0 or over) than with either glucose or lactose. From these data, one can see that there would be little parallelism between blood sugar levels and the height of the R.Q. The authors believed that glucose was changed to a fructose-like substance prior to its combustion in the body.

Very complete studies of the effects of sugar ingestion have been made by Carpenter and his associates. Carpenter and Fox ('30 a) proved the necessity of using 200 cc. or less fluid for the ingestion of dissolved carbohydrates as larger quantities of water increased carbohydrate metabolism as much as 42 per cent and heat production as much as 7 per cent. Continuing their work ('30 b, '30 c) the respiratory exchange of a human subject in the post absorptive state was determined for 3 to 4.5 hours following the ingestion of 5 to 104 gm. of either glucose or fructose. With glucose the R.Q. increased 4 to 6 points reaching a maximum usually the third half hour following ingestion. With fructose, especially the larger quantities, the R.Q. rose more rapidly and to higher levels than noted with glucose. With glucose the heat production averaged 1 calorie for each additional 5 gm. from 5 to 25 gm.,

while in larger amounts it rose more rapidly and in proportion to the additional quantity given. Experiments with levulose revealed increases in heat production in all groups, these varying from 0.5 calorie in 1 hour after 5 gm. levulose to 46 calories in 4 hours after 10 gm.

The effects of galactose ingestion were reported by Carpenter and Lee ('32 a, '32 b). On account of the low threshold, amounts of galactose ranging from 5 to 40 gm. were fed and even with the smaller doses, there was a galactosuria. All amounts caused the R.Q. to rise, the maximum rise in a period varying from 0.04 with 5 gm. to 0.17 with 40 gm. Galactose resembled fructose in its effects on the respiratory quotient and glucose in its effects on heat production. Feeding 20- and 30-gm. doses of galactose to men and women revealed that females had higher maximum rises in respiratory quotient, greater increases in heat production at first after 20 gm., and wider individual deviations in all the features observed. On the other hand, males showed a greater increase in carbohydrate combustion and heat production after 30 gm. or more of the carbohydrate. (See also Deuel, et al., '34.)

Roe, Gilman and Cowgill ('35) pointed out that the species difference in galactose metabolism was due in all probability to the difference in response of the liver and kidneys of the species concerned. Dogs excreted much higher percentages of galactose in the urine than those reported in the literature for humans. The respiratory quotients of normal fasting dogs were not elevated significantly by feeding 2 gm. galactose per kilo. However, if 5 gm. per kilo were fed, there was a delayed elevation of R.Q. characteristic of carbohydrate oxidation. The authors suggest that galactose as such is not oxidized by dogs, rather that galactose is converted into glucose and the latter oxidized, thus causing a significant rise of the quotient. This idea has been supported by data submitted later.

A further study of the effects of sugars fed singly and in various combinations upon respiratory exchange and heat production was made by Carpenter and Lee ('32 c). When glu-

cose and fructose, galactose and fructose, or galactose and glucose were fed in the combinations mentioned, rises in respiratory quotients were noted which agreed with the summation of the rises obtained when the sugars were given separately. With similar combinations of galactose and glucose or fructose, however, there was a greater increase in heat than would be expected from summation effects. It was suggested that the cause might be: 1) the presence of more unabsorbed galactose in the alimentary tract when other sugars were ingested, 2) the greater formation of lactic acid in the intermediary metabolism of galactose, or 3) the smaller formation of glycogen when other glycogen formers were available. Further studies of the effects of glucose and fructose on respiratory metabolism were discussed in another paper (Carpenter and Lee, '33).

It should be noted that Campbell and Maltby ('28) pointed out the danger of using CO_2/O_2 ratios as a means of measuring food transformation, combustion or storage of all carbohydrates. They found that fructose and cane sugar caused a lowering of the CO_2 combining power of the blood and a rise in lactic acid, facts which would account for a stimulation of respiration and an increased elimination of CO_2 and so, a high respiratory quotient. This was not true for glucose, maltose, lactose or galactose. Carpenter and Lee ('33) overruled this objection for fructose, at least, showing that the net rise in R.Q. as a result of its ingestion was due directly to the metabolism of this sugar, for the quantity of organic acids formed could not affect the carbon dioxide tension of alveolar air.

SPECIFIC DYNAMIC ACTION OF CARBOHYDRATES

The specific dynamic action of carbohydrates has been the subject of many investigations. The general conclusion of Deuel and Sandiford ('25) was that all sugars had approximately the same dynamic action. Lusk ('28, '31) presented a comprehensive review of this subject which has been augmented and brought up to date by Wilhelmj ('35), Carpenter

and Fox ('30 b, '30 c) and Carpenter and Lee ('32 a). For detailed information, the reader is referred to these papers.

GLYCOGEN FORMATION BY CARBOHYDRATES

Glycogen formation and storage by the liver have been found to be closely related to the carbohydrate fed, and a review of the older work has been presented in the paper by Feyder and Pierce ('35). A larger amount of liver glycogen was formed from fructose than from glucose according to results obtained by Lusk (1892), who injected the sugars subcutaneously into rabbits. Little glycogen was produced after similar administration of either sucrose or lactose. This raises the question as to whether sucrose must be hydrolyzed prior to building into glycogen. It is likely that hydrolysis of sucrose so given would be slow and as a result not rapidly available for glycogenesis. Rosenfeld ('00) allowed for urinary loss of ingested sugar and concluded that glycogen formation from the sugar retained was greatest for galactose, followed by mannose and glucose.

In early studies no attempt was made to correlate glycogen synthesis with the rate of removal of sugar from the alimentary tract. Sato ('23) injected 2.5 gm. of glucose per kilo intravenously into dogs. The glycogen content of the liver rose slowly reaching a maximum of 2 per cent in 3 hours. Repeated injections of the sugar caused a slower rise in glycogen but a larger total amount. More free sugar was found in the liver than in muscle and no increase in muscle glycogen was noted after the infusion.

Cori ('26) became interested in the relationship between the rate of sugar absorption and glycogenesis in rats. While the absorption of glucose from the intestine was twice as fast for fructose, approximately the same amount of glycogen per 100 gm. of liver was formed from each. Galactose, as a glycogen former, was relatively unimportant. Glycogenesis started slowly after giving the sugars, increased and during the fourth hour started to decrease. The variations in the rate of deposition were not believed by Cori ('26) to be due to an

unequal rate of absorption from hour to hour as he ('25) had shown a constancy of absorption coefficients over a period of hours. The maximum retention of the two sugars 4 hours after feeding, amounted to 17 per cent for glucose and 39 per cent for fructose. Four hours after giving rats either glucose or fructose, Cori and Cori ('28) determined the amount of each oxidized or laid down in the form of liver and body glycogen. Forty-four per cent of the absorbed glucose was oxidized, 18 per cent converted into liver glycogen and 25 per cent into body glycogen. With fructose 36 per cent was oxidized, 38 per cent deposited as liver and 12 per cent as body glycogen. These results showed clearly the necessity of determining glycogen not only in the liver but also in other tissues.

Feeding cornstarch or sucrose at 60 per cent levels in an otherwise balanced diet, Greisheimer and Johnson ('29) found more liver glycogen formed from sucrose than from starch. Further, replacement of part of the sucrose by casein or lard resulted in lessened glycogen deposition. Using diets containing 87.5 per cent of lactose, glucose or sucrose, they ('30) noted the percentage of liver glycogen formed from glucose and sucrose was practically the same, 4.89 per cent, and from lactose but 3.15 per cent. They concluded that if lactose were as readily digested and absorbed as the other two sugars, glycogenesis from all three would be equal. The marked ability of sucrose, as compared with other types of foods, to form glycogen was shown by the results of Greisheimer ('31).

Comparing the rates of liver glycogen formation by glucose, sucrose and corn syrup, and using the Cori ('25) technic, Feyder and Pierce ('35) noted the formation from glucose and sucrose was practically the same during the first and second hours after administration of the sugars, but in the third hour, sucrose formed approximately 44 per cent more glycogen than dextrose. This is in marked contrast with the results of Lusk (1892). Glycogenesis from corn syrup was lower than for the other two sugars, but it proceeded at a fairly uniform rate.

The superiority of sucrose as a glycogen former was assigned to the fructose portion of the molecule. The rate of deposition was independent of the rate of absorption of the sugars.

Although MacKay and Bergman ('33 a) noted no effect of previous diet on glycogen formation in rats fasted 24 or 48 hours before giving glucose, the results of Abelin ('35) showed clearly that state of nutrition was closely related to the amount of glycogen formed from sucrose or starch. There was always a more marked glycogenesis from carbohydrate when the previous diet had been rich in fat.

Recently May ('34) reported that snails, mussels, fish, lower mammals and man contained two animal starches, glycogen and galactogen, the latter being a polymer of galactose. The polysaccharide was not found to any extent in the liver, brain, ovaries or mammary glands, yet was found in large amounts in other tissues of the body. While galactogen was thought to be the mother substance of lactose and brain galactose, the author was not certain as to the sugar giving rise to galactogen. Work of other authors was cited showing that galactogen, or something similar to it, had been found in animal tissues. The significance of the polysaccharide was discussed by May, and his observations open an interesting field for investigation.

FAT FORMATION FROM CARBOHYDRATES

The ability of the body to store glycogen is limited and when carbohydrates are ingested in abundant quantities over a period of time another disposition is made of them, namely, fat formation. The extensive deposits of fat in the adipose tissues of meat animals and the large amounts of milk fat produced by cows, cannot be accounted for as arising solely from the food fat and as far back as the time of Liebig, carbohydrates were considered to be the most suitable raw material for fat synthesis in the animal body.

Many theories were formulated to explain the mechanism of fat formation, some claiming a direct conversion of sugar into fat, others a conversion of sugar into glycogen and a subse-

quent transformation of this storage form into fat. As pointed out by Feyder ('35), several workers have presented data which lead to the inference that glycogen is a mother substance of fat. If this is true, the question arises as to whether the glycogen-forming power of the carbohydrate is in any way related to its fat-forming power. Early work by Rubner (1885) with fasting dogs, showed that to replace 100 gm. of fat, 232 gm. of starch, 234 gm. of sucrose, or 256 gm. of glucose would be required. According to data published by Kellner ('09) starch was a more efficient source of fat than sucrose, 1000 gm. of the former giving rise to 248 gm. whereas an equal amount of the latter produced only 188 gm. The natural conclusion from the above data was that starch and sucrose were equal in fuel value but quite different in their capacity to form fat.

Discussing the ability of sugars to form fat, von Wendt ('23) believed a ketose should not be so effective as an aldose in forming fat because only 3 C atoms would be available. On the other hand, if the modified Magnus-Levy equation (Lusk, '15, '28, p. 350) is used, 4 or 6 carbon atoms would be available.

From the results obtained with an infant, Steuber and Seifert ('28) found that no fat was formed from lactose on a fat-free diet and decided that lactose was not a suitable biological substitute for fat. Later, Jarvis ('30), using rats, published results showing that sucrose formed considerably more fat than lactose and that the latter gave rise to more living tissue. Essentially the same conclusions were drawn by Whittier, Cary and Ellis ('35) from work on rats and pigs.

On comparing the increase in weight of rats in paired feeding experiments giving sucrose or glucose, Feyder ('35) noted more rapid gains after sucrose than after glucose. Further, sucrose fed animals on analysis, contained considerably more fat, but on the average slightly less glycogen than those receiving glucose. As pointed out, it was not probable that the greater fattening effect was due to a lower specific dynamic action, since fructose was known to have a greater

effect on heat production than glucose. Furthermore, Carpenter and Fox ('30) found a higher R.Q. following fructose than glucose ingestion, and believed this was due to the more rapid transformation of fructose into an oxygen poor substance, such as fat. Whether sucrose disposes the animal to reduced activity or glucose stimulates to greater activity, remains to be seen.

SUMMARY

The absorption of carbohydrates from the stomach and intestine of man and animal has been considered. Factors influencing their removal have been presented. The literature reveals that the several sugars have quite different assimilation limits and effects upon blood sugar levels. Likewise, respiration studies demonstrate marked variations in the ease with which the body burns various carbohydrates. The formation of glycogen and fat after the administration of carbohydrates to man and animal has been reviewed.

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